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ORGANOSILICON COMPOUNDS BY  
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AUTHOR(S):

Fukui, Toshiaki

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**STUDIES ON THE BIOCONVERSION OF  
ORGANOSILICON COMPOUNDS  
BY DEHYDROGENASE AND HYDROLASE**

**TOSHIAKI FUKUI**

**1994**

## PREFACE

This is a thesis submitted by the author to Kyoto University for the degree of Doctor of Engineering. The studies collected here have been carried out under the direction of Professor Atsuo Tanaka in the Laboratory of Applied Biological Chemistry, Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, during 1988-1993.

It is the author's great pleasure to express sincere gratitude to Professor Atsuo Tanaka for his continuous guidance and encouragement throughout this work.

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Toshiaki Fukui

Laboratory of Applied Biological Chemistry

Department of Synthetic Chemistry and Biological Chemistry

Faculty of Engineering

Kyoto University

## CONTENTS

Introduction	1
Synopsis	16
Part I Bioconversion of Organosilicon Compounds by Alcohol Dehydrogenase	
Chapter 1.	
Dehydrogenation of trimethylsilylalkanols by horse liver alcohol dehydrogenase: The role of silicon atom in enzymatic reaction	21
Chapter 2.	
Kinetic resolution of trimethylsilylpropanols by enantioselective dehydrogenation with horse liver alcohol dehydrogenase	33
Chapter 3.	
Enantioselective dehydrogenation of $\beta$ -hydroxysilanes by horse liver alcohol dehydrogenase with a novel <i>in situ</i> NAD <sup>+</sup> regeneration system	53



Part II	Bioconversion of Organosilicon Compounds by Hydrolases	
Chapter 1.		
	Efficient kinetic resolution of trimethylsilylpropanols by enantioselective esterification with hydrolases in organic solvent	73
Chapter 2.		
	Chemoenzymatic preparation of optically active silylmethanol derivatives having an asymmetric silicon atom by hydrolase-catalyzed enantioselective esterification	90
General conclusion		117
Publication list		121

## INTRODUCTION

### Enzymes as catalysts in synthesis

Enzymes are proteins having catalytic functions, and many kinds of enzymes conjugately act on the metabolic pathway in living systems. Man has utilized the power of enzymes in living cells, as in fermentation, not only for making of wine and bread but also for production of various metabolites such as amino acids, organic acids, nucleotides, vitamins, and coenzymes.<sup>1-3)</sup>

In recent years, introduction of the functions of enzymes into synthetic processes has been focused on and such an approach is establishing a new area which lies at the border between organic chemistry and biochemistry.<sup>4,7)</sup> Enzymes have following catalytic features in comparison with chemical catalysts.

- 1) Enzymes are highly efficient catalysts. The rates of enzyme-promoted reactions can be faster than those of the corresponding uncatalyzed reactions by factors of up to  $10^{12}$ .<sup>9)</sup>
- 2) Enzymes can work under mild conditions, that is, neutral pH, and normal temperature and pressure. This is one of the advantages for using enzymes as catalysts because this makes it possible to convert compounds which are unstable under severe conditions, and to minimize problems of isomerization, racemization, epimerization, and rearrangement that often

occur in traditional methodology.

- 3) Enzyme-mediated reactions are generally very selective with respect to the structure and stereochemistry of the substrates and products.

This selectivity of enzymes is the basis for much of their utility in synthetic processes.<sup>4,7)</sup> Enzyme-catalyzed reactions are enantioselective, and prochiral stereoselective (selective additions of stereoheterotopic faces, and distinction between enantiotopic or diastereotopic atoms (groups)). Furthermore, enzymes can show chemoselectivity and regioselectivity toward molecules having plural functional groups. Such selectivity is often difficult to achieve by chemical catalysts in spite of recent progress of highly selective reactions in organic chemistry. The use of enzymes as catalysts is, therefore, very effective, especially for synthesis of chiral and multifunctional compounds.

The excellent and useful features of enzymes described above, however, sometimes become disadvantages in the practical use as catalysts; that is, the high selectivity of enzymes is liable to limit the application of enzymes to a narrow field.<sup>5)</sup> Instability of enzymes and inhibition by high concentration of the substrates or products are often observed in enzyme-catalyzed reactions, resulting in inefficient processes.

Much effort has been made to solve these problems, such as screening of new enzymes in nature,<sup>8)</sup> immobilization<sup>9-10)</sup> and chemical modification<sup>11)</sup> of enzymes, and improvement of reaction systems.<sup>12)</sup> Recently, mutagenesis

of proteins using DNA recombination techniques has helped to be understood the relationship between the function and the structure of proteins.<sup>13-14)</sup> These studies will enable to construct efficient processes with the enzymes having favorable catalytic profiles.

### Enzymes in organic solvents

Enzymes have been believed to be inactivated in organic solvents because such solvents disrupt hydrophobic interaction in the molecule and unfold the protein structure. However, recent development of enzyme technology has revealed that enzymes can retain their catalytic functions even in nearly anhydrous organic solvents.<sup>12, 15-19)</sup>

This fact provides a new direction for the application of enzymes. Efficient conversion of lipophilic and water-insoluble compounds with enzymes can be achieved by constructing homogeneous reaction systems using organic solvents. These novel reaction media, in particular, permit hydrolases to catalyze reactions otherwise impossible in water, condensation and group exchange reactions. Furthermore, introduction of organic solvents affects the properties of enzymes, such as thermostability,<sup>20)</sup> substrate specificity,<sup>21)</sup> and enantioselectivity,<sup>22)</sup> indicating the possibility of novel applications of enzyme-catalyzed reactions in organic solvents different from those in water.



### Application of dehydrogenases

Dehydrogenases, which require nicotinamide cofactors (NAD(P)H or NAD(P)<sup>+</sup>) as electron-donors or -acceptors, are the largest and most well-characterized class of oxidoreductases<sup>23)</sup> and very useful enzymes for synthetic purposes due to the ability to catalyze important alcohol/carbonyl oxidoreductions stereoselectively. Especially, horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1) has been widely employed because this enzyme accepts a broad structural range of substrates but retains the high stereoselectivity on each.<sup>4,6)</sup> Asymmetric reduction of various structures of ketones (acyclic<sup>24)</sup> and cyclic<sup>25-26)</sup> has been carried out with HLADH. HLADH can also convert *meso*-diols to highly optically active lactones by asymmetric dehydrogenation with distinction between enantiotopic atoms (groups),<sup>27-28)</sup> and catalyze enantioselective dehydrogenation of racemic alcohols.<sup>29)</sup> The structure of HLADH and the mode of the reaction has been revealed by crystallographic X-ray diffraction analysis,<sup>30)</sup> and a detailed model (cubic space section model) was presented for prediction of the stereochemistry of HLADH-catalyzed oxidoreductions.<sup>28)</sup> Other alcohol dehydrogenases from several sources,<sup>31)</sup> L- and D-lactate dehydrogenases,<sup>32)</sup> and amino acid dehydrogenases<sup>33)</sup> have also been applied for synthesis of optically active compounds.

In dehydrogenase-catalyzed reactions, regeneration of the dissociative cofactors during the reactions is very important because these cofactors are

rather expensive and intrinsically unstable in solution. A lot of works have been devoted to develop various types of regeneration systems for the reduced and oxidized cofactors, including enzymatic and non-enzymatic systems.<sup>4, 34-35)</sup> A suitable regeneration system should be developed or selected to establish an efficient conversion system with dehydrogenases.

### Application of hydrolases

Hydrolases catalyze a wide range of hydrolysis of functional groups including esters, glycosides and anhydrides, as well amides, peptides and other C-N containing functions.<sup>23)</sup> These enzymes are very attractive for synthetic and industrial applications because many of them can be operated stereoselectively with a broad spectrum of substrates and do not require any dissociative cofactors which should be regenerated. Furthermore, many hydrolases are readily available from commercial sources, are generally stable, and consequently, can be easily handled.

Carboxylesterases (EC 3.1.1.1.), and lipases (triacylglycerol acylhydrolases, EC 3.1.1.3.) are the most broadly applied enzymes into synthetic processes.<sup>4,6)</sup> Asymmetric hydrolysis of *meso*-diesters,<sup>36-37)</sup> and enantioselective hydrolysis of racemic esters<sup>38-39)</sup> have been carried out with pig liver esterase and lipases from various sources. These enzymes can also mildly hydrolyze esters which are unstable under the condition of acid- or alkaline-catalyzed hydrolysis, such as prostaglandin esters.<sup>40)</sup>



Lipases are often used in organic solvent systems for conversion of compounds having hydrophobic characters, because the enzymes can be expected to exhibit high degrees of activity in organic solvents due to their hydrolytic ability toward triglycerides at a water-oil interface.<sup>41)</sup> Kinetic resolution of racemic alcohols and acids by enantioselective esterification and transesterification,<sup>42-44)</sup> lactonization,<sup>45)</sup> regioselective acylation of polyols,<sup>46)</sup> and enzymatic synthesis of polyesters<sup>47)</sup> have been carried out using the condensation and group exchange activities of lipases in organic solvents.

A second broadly applicable class of hydrolases is amidases. Kinetic resolution of amino acids by aminoacylase (EC 3.5.1.14) was the first industrial process using immobilized enzymes.<sup>48)</sup> Several kinds of proteases, such as trypsin, chymotrypsin, subtilisin, papain, and thermolysin, not only exhibit amidase and esterase activities but also can catalyze formation of amide and ester bonds under adequate conditions,<sup>49-50)</sup> and synthesis of di- and oligopeptides has been done with these enzymes.<sup>49, 51-52)</sup> One of the advantages in the enzymatic formation of peptide bonds is the unnecessariness to use amino acids having protected side chains as substrates, because side reactions do not occur due to the selectivity of the enzymes.

#### Bioconversion of organosilicon compounds

For wider applications of enzymes, it is necessary to expand the range

of compounds being acceptable for enzymes as substrates, and recent efforts have been focused on the conversion of unconventional compounds by enzymes from this viewpoint. Artificial organometallic compounds are attractive and important targets for bioconversions, because they have larger diversity in their structures and functions than organic compounds in a narrow sense, which are constructed from non-metallic elements (C, H, O, N, S).<sup>53)</sup>

Silicon belongs to the group IVb in the periodic table as does carbon, and has both metallic and non-metallic characters.<sup>54)</sup> The specific characters of silicon compared to carbon are as follows:

- 1) The covalent radius of silicon is longer than that of carbon. The bonds of silicon to carbon and hydrogen are weaker than the corresponding

Table 1. Approximate bond dissociation energies (D) and bond lengths (r) for Si-X and C-X

Bond	D (KJ·mol <sup>-1</sup> )	r (nm)	Bond	D (KJ·mol <sup>-1</sup> )	r (nm)
Si-C	318	0.189	C-C	334	0.153
Si-H	339	0.143	C-H	420	0.109
Si-O	531	0.163	C-O	340	0.141
Si-F	807	0.160	C-F	452	0.139
Si-Cl	472	0.205	C-Cl	335	0.178



bonds between carbon and these elements, whereas its bonds to oxygen and halogen are stronger (Table 1).

- 2) Silicon always appears markedly more electropositive than carbon, resulting in strong polarization of the silicon-carbon bond (Table 2).
- 3) Silicon not only constructs quadricovant compounds with tetrahedral  $sp^3$ -hybridized bonds similar to carbon, but also does hypercovalent compounds because of its vacant  $d$ -orbital.

Owing to these fundamental differences, organosilicon compounds possessing silicon-carbon bonds show unique chemical and physical properties, and play important roles in synthetic chemistry and chemical industry, as versatile synthetic equivalents of active species in highly selective synthesis, and new functional materials, and their precursors.<sup>55-56)</sup>

Recently, silicon has also been recognized as an important element for the biosphere, for example, it participates in the normal metabolism of higher animals and plants.<sup>57)</sup> Although silicon is the second most abundant

Table 2. Relative electronegativity

H	B	C	N	O	F
2.79	1.84	2.35	3.16	3.52	4.0
	Al	Si	P	S	Cl
	1.40	1.64	2.11	2.52	2.84

element in the Earth's crust, silicon compounds are mainly associated with minerals, soil, and other non-living systems as compounds bonded to four oxygen atoms, and the biochemical processes with silicon seemed to involve such inorganic silicon compounds. The organosilicon compounds have not been detected in living systems whereas organic carbon compounds are well known as integral parts of all living matters.

Since the discovery of the high biological potential of several classes of organosilicon compounds, the number of studies concerning pharmacological and toxicological investigations of bioactive organosilicon species have been expanded rapidly.<sup>58-60)</sup> However, no fundamental studies have been done on the mode of the recognition and reaction of enzymes toward the organosilicon compounds as non-natural substrates in comparison with conventional substrates. It is very interesting to investigate the interaction between enzymes and the organosilicon compounds in order to see whether the effects of the silicon atom toward enzymes are detectable or not.

Furthermore, only a few results have been reported on bioconversion of organosilicon compounds.<sup>61-63)</sup> Production of organosilicons with desired chemical and physical properties by employing enzymes is of great importance from an industrial viewpoint. For example, preparation of optically active organosilicon compounds by utilizing the selectivity of enzymes is very attractive for the advanced application of enzymes. In recent years there has been increasing interest in the optically active organosilicon species with



respect to their use as reagents in asymmetric synthesis<sup>64-65)</sup> and as drugs in experimental pharmacology,<sup>59-60)</sup> and several methods have been developed to prepare them including resolution through separation of diastereomeric derivatives, kinetic resolution, asymmetric synthesis, and stereospecific synthesis starting from chiral compounds.<sup>66-67)</sup> Stereoselective conversion of organosilicon compounds catalyzed by enzymes will provide new methods for preparation of the optically active organosilicon compounds, and the effect of the silicon atom on the stereoselectivity of enzymes is also interesting to be studied.

In this thesis, conversion of organosilicon compounds by horse liver alcohol dehydrogenase (HLADH) and hydrolases is attempted. The effects of the silicon atom in substrates on the recognition and reaction of these enzymes were investigated in comparison with the corresponding carbon compounds. Furthermore, preparation of optically active organosilicon compounds, having a chiral center not only on the carbon atom but also on the silicon atom, was carried out by enantioselective reaction with enzymes.

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## SYNOPSIS

### PART I Bioconversion of Organosilicon Compounds by Alcohol Dehydrogenase

The use of unconventional compounds as substrates for enzymes is current research in biotechnology to increase the potential of and expand the application of enzymes. Introduction of organosilicon compounds into enzymatic reactions as non-natural substrates is considered to give not only interesting information for the recognition and reaction of enzymes, but also important procedures for production of useful organosilicons, such as optically active organosilicon compounds.

This part deals with the bioconversion of organosilicon compounds by alcohol dehydrogenase (ADH). ADH has been proved to be a very versatile enzyme for the conversion of organic compounds because of its ability to catalyze important alcohol/carbonyl oxidoreductions stereoselectively.

In Chapter 1, trimethylsilylalkanols ( $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$ ,  $n=1-3$ ) were examined as substrates for ADH, and horse liver alcohol dehydrogenase (HLADH) was found to exhibit a dehydrogenation activity toward 2-trimethylsilylethanol ( $n=2$ ) and 3-trimethylsilylpropanol ( $n=3$ ). 2-Trimethylsilylethanol was a better substrate for HLADH than its carbon

analogue. However, trimethylsilylmethanol ( $n=1$ ) did not serve as a substrate in spite of fairly high activity of the enzyme toward its carbon analogue, in contrast. The reason of the improved activity of HLADH in the case of 2-trimethylsilylethanol was revealed due to its higher affinity toward the enzyme and the lower activation energy of the reaction compared to the carbon counterpart, derived from the bigger atomic radius and the lower electronegativity of the silicon atom than those of the carbon atom. The inactivity of HLADH toward trimethylsilylmethanol could be also explained by the electric effect of the silicon atom.

In Chapter 2, HLADH-catalyzed enantioselective dehydrogenation of three isomers of racemic trimethylsilylpropanols was tried. HLADH showed a dehydrogenation activity toward 1-trimethylsilyl-2-propanol and 2-trimethylsilyl-1-propanol in a water-*n*-hexane two-layer system with coenzyme regeneration using reductive amination of 2-oxoglutarate by L-glutamate dehydrogenase. Especially, 1-trimethylsilyl-2-propanol could be resolved with high optical purity (>99 %ee) by this system. The silicon atom in the substrates was found to enhance the enantioselectivity of the enzyme, and this phenomenon could be explained by the assumption that the more bulky and/or hydrophobic trimethylsilyl group would facilitate to recognize the chirality for HLADH. Kinetic analysis in an aqueous monolayer system demonstrated that the specific properties of the silicon atom also greatly affected the dehydrogenation reaction, such as the higher affinity of



the organosilicon compounds toward HLADH than that of their corresponding carbon compounds.

Chapter 3 deals with a novel *in situ* coenzyme regeneration system in the HLADH-catalyzed dehydrogenation based on a property of organosilicon compounds. On the dehydrogenation of racemic 2-trimethylsilyl-1-propanol, the reaction proceeded with only HLADH and a catalytic amount of  $\text{NAD}^+$ , and the (-)-enantiomer was remained in the reaction mixture. Stoichiometric analysis proved that the  $\beta$ -carbonylsilane, produced selectively from the (+)-enantiomer by HLADH, was spontaneously degraded by addition of water into trimethylsilanol and *n*-propanal, and then,  $\text{NAD}^+$  was regenerated through HLADH-catalyzed reduction of *n*-propanal to *n*-propanol. On the contrary, dehydrogenation of the carbon counterpart was negligible under the same conditions because of stability of the dehydrogenated product. Other primary  $\beta$ -hydroxysilanes having different substituents on the chiral center or on the silicon atom were also enantioselectively dehydrogenated by HLADH with the *in situ* coenzyme regeneration, and the chiral recognition of HLADH toward primary alcohols was also discussed.

## **PART II Bioconversion of Organosilicon Compounds by Hydrolases**

This part deals with the bioconversion of organosilicon compounds by hydrolases. Hydrolases are useful enzymes for synthetic and industrial

purposes because many of them can stereoselectively convert substrates having diverse structures and be easily handled without any dissociative cofactors.

In Chapter 1, kinetic resolution of three isomers of racemic trimethylsilylpropanols was tried by enantioselective esterification with five kinds of hydrolases in an organic solvent system. The hydrolases were found to be able to esterify these organosilicon compounds with 5-phenylpentanoic acid, even  $\beta$ -hydroxysilanes which are unstable under the conditions of acid-catalyzed esterification. The highly optically pure trimethylsilylpropanols including a primary alcohol were successfully prepared with the selected hydrolases. Comparative studies were made by using their carbon counterparts and it was revealed that the silicon atom in the substrates often enhanced the enantioselectivity of the hydrolases, but the effect of the silicon atom on the reactivity of the substrates was dependent on their structure. The results obtained were rationalized based on the characteristics of the silicon atom.

Chapter 2 deals with chemoenzymatic preparation of optically active silylmethanol derivatives having an asymmetric silicon atom by enantioselective esterification with hydrolases. Twenty kinds of hydrolases were examined on esterification of chemically synthesized racemic ethylmethylphenylsilylmethanol with 5-phenylpentanoic acid in an organic solvent, and only one enzyme, a commercial crude papain preparation was

found to exhibit a high enantioselectivity with moderate activity on the esterification, and the highly optically pure (+)-enantiomer (92 %ee) could be obtained with this enzyme. From investigation using analogues of the silicon-containing alcohol, a short methylene chain between the silicon atom and the hydroxyl group, and an aromatic substituent on the silicon atom were revealed to be essential for the high activity and high enantioselectivity of crude papain. Several silylmethanol derivatives having an asymmetric silicon atom could be also resolved highly enantioselectively by this reaction system, even though chemical synthesis of such chiral quaternary silanes with high optical purity was generally difficult or very complicated. Enzymatic methods were proved to be useful for preparation of optically active silanes.

## **PART I Bioconversion of Organosilicon Compounds by Alcohol Dehydrogenase**

### **Chapter 1. Dehydrogenation of trimethylsilylalkanols by horse liver alcohol dehydrogenase: The role of silicon atom in enzymatic reaction**

#### **INTRODUCTION**

Recently, interest in the use of unconventional compounds as substrates of enzymes has been risen, thereby increasing the potential and expanding the use of enzymes. For example, bioconversion of organosilicon compounds is of great interest both in the fundamental study of enzymology and in the production of useful organosilicon compounds because they play important roles in synthetic organic chemistry.<sup>1-2)</sup> Kawamoto *et al.*<sup>3)</sup> have reported the use of organosilicon compound, trimethylsilylmethanol, as an efficient unconventional substrate for enantioselective esterification of 2-(4-chlorophenoxy)propanoic acid with yeast lipase in organic media. In this case, the silicon atom served as a more effective atom than the carbon atom to enhance the activity of this enzyme.

Furthermore, bioconversion of organosilicon compounds by alcohol dehydrogenase (ADH) is very attractive because of the importance of



asymmetric alcohol/carbonyl oxidoreduction in organic chemistry.<sup>4-6)</sup> Although a few papers are available on the reduction of organosilicon compounds by microorganisms,<sup>7-8)</sup> it is not clear whether ADH can actually catalyze the oxidoreduction of organosilicons in these organisms and it has not been possible to discuss the effects of the silicon atom on enzymatic reaction with ADH.

In this chapter, three preparations of ADH were examined for dehydrogenation of organosilicon compounds,  $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$  ( $n=1-3$ ). The effects of the silicon atom in these compounds on horse liver alcohol dehydrogenase (HLADH)-catalyzed reaction were discussed on the basis of the physical properties of the silicon atom compared to the corresponding carbon compounds,  $\text{Me}_3\text{C}(\text{CH}_2)_n\text{OH}$  ( $n=1-2$ ) (Fig.1).

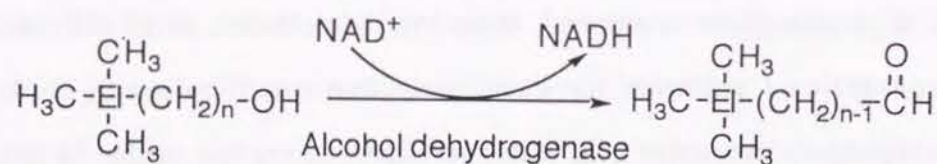


Fig. 1. ADH-catalyzed dehydrogenation reaction. El= Si or C.

## MATERIALS AND METHODS

### Materials

HLADH and yeast alcohol dehydrogenase (YADH) were purchased from Sigma, St. Louis, MO, USA. Another YADH and  $\beta$ -nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) were obtained from Oriental Yeast, Tokyo, Japan. Trimethylsilylmethanol was a product of Shin-Etsu Kagaku, Tokyo, Japan. 2-Trimethylsilylethanol, 3-trimethylsilylpropanol, and 3,3-dimethylbutanol were purchased from Aldrich, Milwaukee, WI, USA and 2,2-dimethylpropanol from Nacalai Tesque, Kyoto, Japan. Other chemicals were also obtained from commercial sources.

### Alcohol dehydrogenase assay

The assay mixture (3.3 ml) containing 15 mM substrate, 0.588 mM  $\text{NAD}^+$ , and 5 % tetrahydrofuran (THF) in 50 mM Tris-HCl buffer (PH 8.8) was preincubated at 30 °C for 3 min and 100  $\mu\text{l}$  of enzyme solution (1  $\text{mg}\cdot\text{ml}^{-1}$ ) was added. The reaction rate was calculated from the increase in absorbance of NADH at 340 nm ( $\Delta\epsilon_{340} = 6.22 \times 10^3 \text{ M}^{-1}\cdot\text{cm}^{-1}$ ) measured by a Shimadzu MPS-200 spectrophotometer.

### Measurement of kinetic parameters

Enzymatic activity of HLADH at different substrate concentrations



was measured spectrophotometrically at 30 °C. The assay mixture (3.4 ml) contained 1.2 mM NAD<sup>+</sup>, 5 % THF and 0.408 mg HLADH in the buffer with varying concentrations of the substrate from 1 mM to 6 mM. A  $C_s \cdot V^{-1}$  versus  $C_s$  plot was used to obtain the kinetic parameters, where  $C_s$  is the initial substrate concentration and  $V$  is the initial reaction rate.

#### **Measurement of activation energy ( $E_A$ ) and frequency factor ( $A$ )**

The activity of HLADH on 2-trimethylsilylethanol and 3,3-dimethylbutanol at different temperatures (20-30 °C) were measured. The assay mixture (3.4 ml) contained 1.2 mM NAD<sup>+</sup>, 5 % THF, 0.408 mg HLADH and 0.75 mM substrate in the buffer. A  $\ln k$  versus  $T^{-1}$  plot was used to obtain  $E_A$  and  $A$ , where  $k$  is the reaction rate coefficient and  $T$  is the absolute temperature.

## **RESULTS**

#### **Screening of ADH**

HLADH and YADH (from Sigma and Oriental Yeast) were tested for the dehydrogenation of three organosilicon compounds ( $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$ ,  $n=1-3$ ). HLADH showed a enzymatic activity on two of them, 2-trimethylsilylethanol ( $n=2$ ) and 3-trimethylsilylpropanol ( $n=3$ ) (Table 1), while yeast enzymes did not act on them under the conditions employed.

Table 1. Activity of HLADH on trimethylsilylalkanols and their carbon analogues

Alcohol	$n^a$	Relative activity <sup>b</sup> (%)
Ethanol	-	100
Trimethylsilylmethanol	1	0
2-Trimethylsilylethanol	2	209
3-Trimethylsilylpropanol	3	145
2,2-Dimethylpropanol	1	75
3,3-Dimethylbutanol	2	146

<sup>a</sup> Number of methylene group(s) between the trimethylsilyl/*tert*-butyl group and the hydroxyl group.

<sup>b</sup> The reaction rate on ethanol was expressed as 100 %. The activity was measured spectrophotometrically at a fixed substrate concentration.

The organosilicon compounds were found to be the substrates of HLADH.

#### **Enzymatic activity of HLADH**

Table 1 shows the activity of HLADH on the three organosilicon compounds having different chain length between the silicon atom and the hydroxyl group ( $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$ ,  $n=1-3$ ) and their carbon counterparts ( $\text{Me}_3\text{C}(\text{CH}_2)_n\text{OH}$ ,  $n=1-2$ ). 2-Trimethylsilylethanol ( $n=2$ ) and 3-trimethylsilylpropanol ( $n=3$ ) were better substrates for HLADH than ethanol, but

trimethylsilylmethanol ( $n=1$ ) was not a substrate. To clarify the effect of the silicon atom, the organosilicon compounds were compared with the corresponding carbon compounds. In the case of  $n=2$ , 2-trimethylsilylethanol was a better substrate than its carbon counterpart, 3,3-dimethylbutanol. However, trimethylsilylmethanol was inert in contrast to its carbon analogue, 2,2-dimethylpropanol. The influence of the silicon atom on the reactivity of the substrates was quite different depending on the number of methylene group(s),  $n$ .

#### Kinetic parameters

The effect of the silicon atom on the kinetic parameters,  $K_m$  and  $V_{max}$ , of the HLADH-catalyzed dehydrogenation reaction was also studied. The results obtained were shown in Table 2. The value of  $V_{max}$  increased with the decrease in  $K_m$ , and the replacement of the carbon atom in 3,3-dimethylbutanol by the silicon atom decreased the value of  $K_m$  and increased that of  $V_{max}$ . That is to say, this replacement by the silicon atom caused the increase in affinity of HLADH toward the substrate, and the improvement of the reactivity. According to the value of  $K_m$ , 2-trimethylsilylethanol had the strongest affinity toward HLADH among all of the substrates examined, giving the highest activity of HLADH.

Table 2. Values of  $K_m$  and  $V_{max}$  of HLADH-catalyzed dehydrogenation reaction

Alcohol	$n^a$	$K_m$ (mM)	$V_{max}$ ( $\mu\text{mol} \cdot \text{min}^{-1}$ )
Ethanol	-	1.55	0.194
2-Trimethylsilylethanol	2	0.520	0.360
3-Trimethylsilylpropanol	3	0.881	0.268
2,2-Dimethylpropanol	1	3.40	0.148
3,3-Dimethylbutanol	2	0.773	0.283

<sup>a</sup> Number of methylene group(s) between the trimethylsilyl/*tert*-butyl group and the hydroxyl group.

#### Activation energy ( $E_A$ ) and frequency factor ( $A$ )

For more comprehensive study for the effect of the silicon atom on the reaction of organosilicon compounds by HLADH,  $E_A$  and  $A$  of the HLADH-catalyzed dehydrogenation reaction were measured. The dehydrogenation of 2-trimethylsilylethanol by HLADH had lower  $E_A$  and  $A$  values than those of 3,3-dimethylbutanol (Table 3). These results indicate that the replacement of the carbon atom in 3,3-dimethylbutanol by the silicon atom decreased the activation energy and frequency factor. As  $E_A$  influences the reaction rate exponentially, it plays a much more important role in the reactivity of the substrate than  $A$  in this case. Therefore, the



Table 3. Activation energy ( $E_A$ ) and frequency factor (A) of HLADH-catalyzed dehydrogenation reaction

Alcohol	$n^a$	$E_A$ (J·mol <sup>-1</sup> ·K <sup>-1</sup> )	A	$R^b$
2-Trimethylsilylethanol	2	$2.07 \times 10^4$	1.11	0.997
3,3-Dimethylbutanol	2	$2.55 \times 10^4$	6.07	0.996

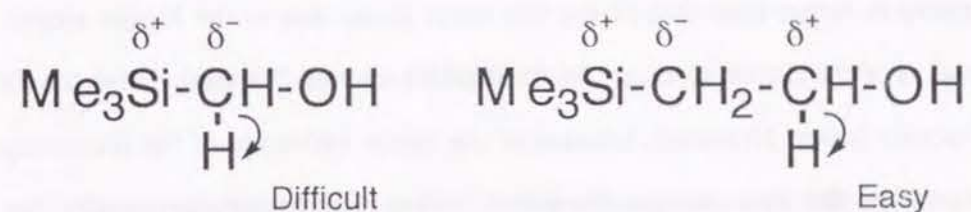
<sup>a</sup> Number of methylene group(s) between the trimethylsilyl/*tert*-butyl group and the hydroxyl group.

<sup>b</sup> Correlation coefficient.

enzymatic activity on 2-trimethylsilylethanol became higher than that on 3,3-dimethylbutanol, even though the A of the carbon compound was higher.

## DISCUSSION

It is well known that the silicon atom has the low electronegativity and can stabilize the negative charge at  $\alpha$ -position and the positive charge at  $\beta$ -position of the carbon atom from the silicon atom.<sup>1)</sup> Owing to the lower electronegativity of the silicon atom, the electron density around the  $\alpha$ -carbon atom of trimethylsilylmethanol ( $n=1$ ) is higher than that of 2,2-dimethylpropanol. This makes it more difficult for the  $H^-$  anion to be removed from the  $\alpha$ -carbon atom, which is the first step of the dehydrogenation of alcohol



(A) Trimethylsilylmethanol

(B) 2-Trimethylsilylethanol

Fig. 2. Electrical effects of the silicon atom on removal of the  $H^-$  anion from the carbon atom.

catalyzed by ADH<sup>9</sup> (Fig. 2A). As a result, the activation energy of the dehydrogenation of trimethylsilylmethanol by HLADH probably becomes considerably higher than that of 2,2-dimethylpropanol, and thus trimethylsilylmethanol is not a substrate, while its carbon analogue is a good one. Conversely, as the silicon atom stabilizes the positive charge on the  $\beta$ -carbon atom, the  $H^-$  anion could be removed from the  $\beta$ -carbon atom of 2-trimethylsilylethanol ( $n=2$ ) more easily than from that of 3,3-dimethylbutanol (Fig. 2B). This can be supported by the observed lower activation energy of the dehydrogenation of 2-trimethylsilylethanol by HLADH. In the case of 3-trimethylsilylpropanol ( $n=3$ ), the effect of the silicon atom will become weaker because of the long distance between the silicon atom and the

carbon atom binding the hydroxyl group. Steric hindrance of the trimethylsilyl group is larger than that of the *tert*-butyl group due to the bigger atomic radius of the silicon atom, so the frequency factor of 2-trimethylsilylethanol became lower. However, because of the minor influence of the frequency factor on the reaction rate compared to that of the activation energy, the enzymatic activity of HLADH on 2-trimethylsilylethanol was higher than that on 3,3-dimethylbutanol, even though the frequency factor of the carbon counterpart was higher.

The structure of HLADH has been determined crystallographically and it has been revealed that HLADH has a hydrophobic active center when NAD<sup>+</sup> is bound.<sup>9-10</sup> The higher affinity of HLADH toward 2-trimethylsilylethanol is probably due to the higher hydrophobicity of the trimethylsilyl group<sup>11</sup> and also to the appropriate distance between the trimethylsilyl group and the hydroxyl group derived from the bigger radius of the silicon atom.

YADH could dehydrogenate only ethanol among the six compounds used in this study under the conditions employed. These results indicate that YADH can not accept alcohols having a bulky group, such as trimethylsilyl and *tert*-butyl.

In conclusion, it has been found in this study that HLADH is able to catalyze the dehydrogenation of organosilicon compounds, and that the silicon atom in substrates greatly affected to the enzymatic reaction. The effects of the silicon atom could be explained in terms of its specific characters,

which are the low electronegativity and the big atomic radius.

## SUMMARY

Bioconversion of three organosilicon compounds with different chain length between the silicon atom and the hydroxyl group ( $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$ ,  $n=1-3$ ) by horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1.) was studied. Furthermore, the effects of the silicon atom on the HLADH-catalyzed reaction were examined in comparison with the corresponding carbon compounds. HLADH could catalyze the dehydrogenation of 2-trimethylsilylethanol ( $n=2$ ) and 3-trimethylsilylpropanol ( $n=3$ ). 2-Trimethylsilylethanol was a better substrate than both its carbon analogue, 3,3-dimethylbutanol, and ethanol. The improved activity of HLADH on 2-trimethylsilylethanol could be accounted for by the higher affinity toward HLADH and the lower activation energy of the reaction with HLADH than those of the carbon counterpart. These are derived from the physical properties of the silicon atom, that is, the lower electronegativity and the bigger atomic radius than those of the carbon atom. In contrast, HLADH showed no activity on trimethylsilylmethanol ( $n=1$ ), whereas it catalyzed the dehydrogenation of the carbon analogue, 2,2-dimethylpropanol, fairly well. The reason for the inactivity of HLADH in the case of trimethylsilylmethanol was discussed based on the electric effect of the silicon atom.



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## Chapter 2. Kinetic resolution of trimethylsilylpropanols by enantioselective dehydrogenation with horse liver alcohol dehydrogenase

### INTRODUCTION

An active area of current research in biotechnology is the use of unconventional compounds as substrates for enzymes to expand the application of enzymes. The author has selected organosilicon compounds as non-natural substrates for horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1.) because of their unique characteristics.<sup>1-3)</sup>

HLADH has proved to be one of the most versatile enzymes, being promising for conversion of organic compounds, because it catalyzes alcohol/carbonyl oxidoreduction of a broad spectrum of substrates with high degrees of stereoselectivity.<sup>4)</sup> However, no work had been done on the bioconversion of organosilicon compounds with HLADH until the author's study, which demonstrated that HLADH was capable of catalyzing the dehydrogenation of organosilicon compounds,  $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$  ( $n=1-3$ ), and that the silicon atom in the structure had a great effect on the activity of HLADH (PART I, Chapter 1).<sup>5)</sup>

As a further step toward the study on bioconversion of organosilicon compounds, this chapter deals with the enantioselective dehydrogenation of

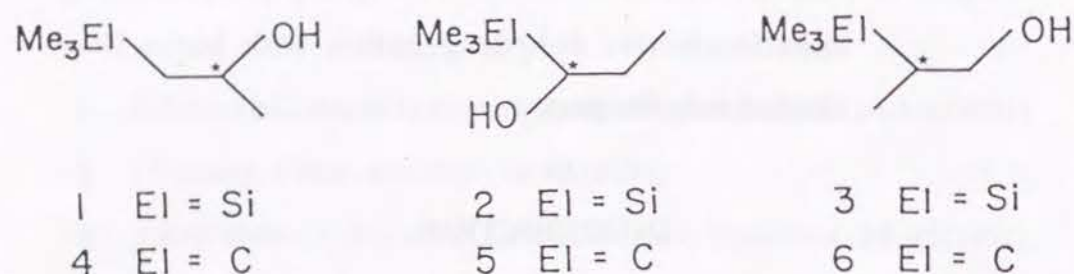


Fig. 1. Structures of three isomers of racemic trimethylsilylpropanols and their carbon analogues used as substrates for HLADH.

three isomers of racemic trimethylsilylpropanols (Fig. 1, **1-3**) catalyzed by HLADH in a two-layer system with coenzyme regeneration. The production of interesting organosilicons, such as optically active organosilicon compounds, by utilizing the selectivity of enzymes is also very important. Furthermore, the effects of the silicon atom on the activity and enantioselectivity of HLADH were investigated in comparison with their carbon counterparts (Fig. 1, **4-6**), and the results obtained were rationalized on the basis of the characteristics of the silicon atom and the structure of HLADH.

## MATERIALS AND METHODS

### Analyses

$^1\text{H-NMR}$  spectra were measured with a Varian Gemini-200 NMR spectrometer and IR spectra with a JASCO IR-810 spectrometer. GLC analyses were carried out using a Shimadzu GC-12A equipped with a flame-ionization detector and HPLC analyses were done using a Hitachi L-6000 instrument equipped with an L-4200 UV-Vis detector. A Shimadzu MPS-200 spectrophotometer was used for kinetic analysis. Specific rotations were determined with a JASCO DIP-140 polarimeter.

### Materials

HLADH and 2-oxoglutarate (disodium salt) were purchased from Sigma, St. Louis, MO, USA and L-glutamate dehydrogenase (GIDH) from Biozyme, South Wales, UK.  $\beta$ -Nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) was obtained from Oriental Yeast, Tokyo, Japan. 1-Trimethylsilyl-2-propanol (**1**),<sup>6</sup> 1-trimethylsilyl-1-propanol (**2**),<sup>7</sup> and 2-trimethylsilyl-1-propanol (**3**)<sup>7</sup> were donated by Nitto Denko, Osaka, Japan. 4,4-Dimethyl-2-pentanol (**4**) and 2,2-dimethyl-3-pentanol (**5**) were purchased from Aldrich, Milwaukee, WI, USA. 2,3,3-Trimethyl-1-butanol (**6**) was prepared as follows. (*R*)-methoxytrifluoromethylphenylacetic acid (MTPA) was a product of Nacalai Tesque, Kyoto, Japan. Other chemicals were also obtained from commercial sources.

### 2,3,3-Trimethyl-1-butanol (**6**)



2,3,3-Trimethyl-1-butene (3.5 g, 36 mmol) (Tokyo Kasei, Tokyo, Japan) was dropped into a 0.3 l flask containing 80 ml of 0.5 M 9-borabicyclo[3,3,1]nonane<sup>®</sup> in tetrahydrofuran (Aldrich) under an N<sub>2</sub> atmosphere, followed by stirring at room temperature for further 1 h. Then water (5 ml), 3N NaOH (17 ml), and 30 % H<sub>2</sub>O<sub>2</sub> (17 ml) were added successively at controlled temperature (less than 50 °C) and the aqueous phase was saturated with K<sub>2</sub>CO<sub>3</sub> after refluxing for 1 h. The organic phase, together with the ethyl ether extract of the aqueous phase, was dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and distilled under atmospheric pressure. The bp 130-140 °C fraction was collected to give **6** as colorless oil (3.0 g, 72 %). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ 0.87 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.94 (d, *J*=6.8 Hz, 3H, CH<sub>3</sub>), 1.34 (s, 1H, OH), 1.36-1.42 (m, 1H, CH), 3.32 (dd, *J*=8.7, 10.4 Hz, 1H, CH<sub>2</sub>), 3.81 (dd, *J*=3.9, 10.4 Hz, 1H, CH<sub>2</sub>); IR (neat) 3350, 2950, 1470, 1380, 1030 cm<sup>-1</sup>.

#### *Enantioselective dehydrogenation by HLADH in a two-layer system*

Racemic substrate (100 μmol) shown in Fig. 1 was dissolved in water-immiscible organic solvent (10 ml) and mixed with 50 mM Tris-HCl buffer (pH 8.8, 10 ml) containing 10 IU HLADH, 20 mM 2-oxoglutarate (disodium salt), 20 mM ammonium acetate, and 20 IU GIDH. After stirring at 30 °C for 30 min, 0.2 μmol NAD<sup>+</sup> was added to start the reaction and the mixture was stirred at 30 °C. It was confirmed that only a small amount of the

substrate was present in the water phase. The conversion ratio was expressed as the decrease in substrate concentration in the organic phase measured by GLC using a glass column (diameter 3.0 mm x 1.0 m) packed with PEG-HT supported on Uniport R (GL Sciences, Tokyo, Japan) (carrier gas, N<sub>2</sub>; flow rate, 60 ml·min<sup>-1</sup>). *n*-Pentadecane was used as the internal standard. The optical purity of the remaining substrates was determined with HPLC using a Sumichiral OA-4600 column (diameter 4.0 mm x 250 mm, Sumika Chemical Analysis Service, Osaka, Japan) after derivatization with 3,5-dinitrophenyl isocyanate<sup>9</sup> (Sumika Chemical Analysis Service). The mobile phase was *n*-hexane/2-propanol (100:1 v/v) for analysis of **1** and **4**. For **3** and **6**, two columns of Sumichiral OA-4600 in series were used and the mobile phase was *n*-hexane/2-propanol (98:2 v/v). The flow rate was 1.0 ml·min<sup>-1</sup> and the eluent was monitored at 254 nm in both cases. The enantiomeric excess (%ee) was calculated from peak areas of both enantiomers in the remaining alcohols.

#### *Determination of absolute configuration*

The absolute configurations of **1** and **4** were determined by correlation method with <sup>1</sup>H-NMR.<sup>10)</sup> (+)-**1** and (+)-**4** were prepared by enantioselective esterification of racemic **1** and **4** with 5-phenylpentanoic acid and lipoprotein lipase (PART II, Chapter 1).<sup>11)</sup> Diastereomeric esters of (+)-**1**, (+)-**4**, and their racemic compounds were synthesized with (*R*)-MTPA and analyzed



by  $^1\text{H-NMR}$ . The configuration of each enantiomer was determined from the observed chemical shifts. The spectra data were as follows:

[(*R*)-MTPA] ester of (*S*)-(+)-**1**.  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  -0.019 (s, 9H,  $\text{Si}(\text{CH}_3)_3$ ), 0.91 (dd,  $J=9.6, 14.1$  Hz, 2H,  $\text{CH}_2$ ), 1.36 (d,  $J=6.3$  Hz, 3H,  $\text{CH}_3$ ), 3.56 (q,  $J=1.2$  Hz, 3H,  $\text{OCH}_3$ ), 5.2-5.3 (m, 1H, CH), 7.4-7.6 (m, 5H,  $\text{C}_6\text{H}_5$ ).

[(*R*)-MTPA] ester of (*R*)-(-)-**1**.  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.041 (s, 9H,  $\text{Si}(\text{CH}_3)_3$ ), 1.00 (dd,  $J=10.1, 13.9$  Hz, 2H,  $\text{CH}_2$ ), 1.28 (d,  $J=6.2$  Hz, 3H,  $\text{CH}_3$ ), 3.54 (q,  $J=1.4$  Hz, 3H,  $\text{OCH}_3$ ), 5.2-5.3 (m, 1H, CH), 7.4-7.6 (m, 5H,  $\text{C}_6\text{H}_5$ ).

[(*R*)-MTPA] ester of (*S*)-(+)-**4**.  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.078 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.33 (d,  $J=6.1$  Hz, 3H,  $\text{CH}_3$ ), 1.39 (d,  $J=3.4$  Hz, 2H,  $\text{CH}_2$ ), 3.57 (q,  $J=1.5$  Hz, 3H,  $\text{OCH}_3$ ), 5.1-5.3 (m, 1H, CH), 7.4-7.6 (m, 5H,  $\text{C}_6\text{H}_5$ ).

[(*R*)-MTPA] ester of (*R*)-(-)-**4**.  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.095 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.26 (d,  $J=6.1$  Hz, 3H,  $\text{CH}_3$ ), 1.43 (d,  $J=4.0$  Hz, 2H,  $\text{CH}_2$ ), 3.50 (q,  $J=1.3$  Hz, 3H,  $\text{OCH}_3$ ), 5.1-5.3 (m, 1H, CH), 7.4-7.6 (m, 5H,  $\text{C}_6\text{H}_5$ ).

#### *Kinetic analysis in an aqueous monolayer system*

The assay mixture (3.3 ml) containing various concentrations of the substrate, 1.2 mM  $\text{NAD}^+$ , and 1 % tetrahydrofuran in 50 mM Tris-HCl buffer (pH 8.8) was preincubated at 30 °C for 3 min and then 0.4 ml HLADH solution ( $3.4 \text{ IU}\cdot\text{ml}^{-1}$ ) was added. The reaction rate was calculated

from the increase in absorbance of NADH at 340 nm ( $\Delta\epsilon_{340} = 6.22 \times 10^3 \text{ M}^{-1}\cdot\text{cm}^{-1}$ ). A  $\text{Cs}\cdot\text{V}^{-1}$  versus Cs plot was used to obtain the values of  $K_m$  and  $V_{\text{max}}$ , where Cs is the initial substrate concentration and V is the initial reaction rate.

## RESULTS

#### *Enantioselective dehydrogenation by HLADH in a two-layer system*

Enantioselective dehydrogenation of the three isomers of racemic trimethylsilylpropanols (**1-3**) with HLADH was tried by examining the effect of the silicon atom on the enantioselectivity of HLADH compared to their carbon analogues (**4-6**). Product inhibition is generally a big problem in oxidation reactions catalyzed by alcohol dehydrogenases.<sup>9</sup> To solve this problem, a water-organic solvent two-layer system was applied with coenzyme regeneration (oxidation of NADH to  $\text{NAD}^+$  coupled with GIDH-catalyzed reductive amination of 2-oxoglutarate to L-glutamate)<sup>12</sup> (Fig. 2). *n*-Hexane was found to be the best among the organic solvents tested (chloroform, 1,2-dichloroethane, ethyl acetate, and *n*-hexane).

The time-course of the HLADH-catalyzed dehydrogenation of **1** and **3**, and their carbon counterparts, **4** and **6**, was followed in the water-*n*-hexane two-layer system with coenzyme regeneration (Fig. 3). With **1**, the reaction proceeded quickly and stopped at 50 % conversion, whereas the carbon

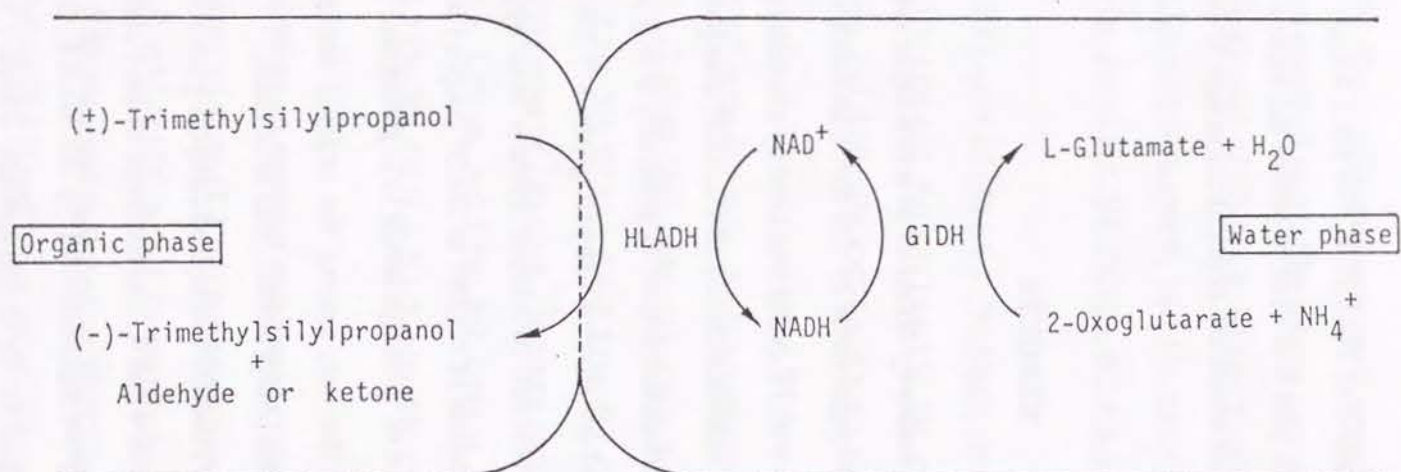


Fig. 2. A two-layer system for HLADH-catalyzed enantioselective dehydrogenation of organosilicon compounds with coenzyme regeneration. GLDH, L-glutamate dehydrogenase.

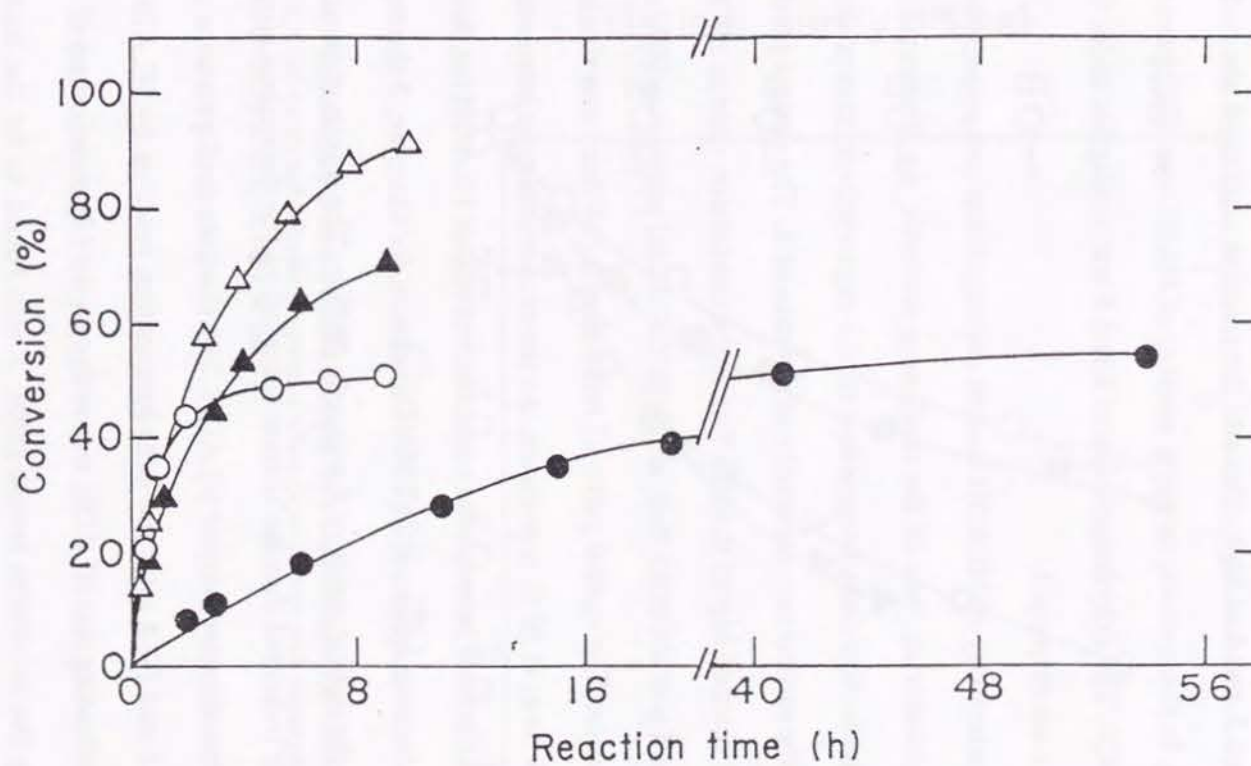


Fig. 3. Time-course of HLADH-catalyzed dehydrogenation of the organosilicon compounds and their carbon analogues in a two-layer system with coenzyme regeneration. Symbols: (○), 1; (△), 3; (●), 4; (▲), 6.



analogue **4** reacted slowly and the conversion reached over 50 %. HLADH dehydrogenated **3** and **6** at high rates and the reaction continued above 50 % conversion. In these cases, turnover number of  $\text{NAD}^+$  was calculated to be more than 400. The dehydrogenation of **2** and **5** was negligible under the reaction conditions employed.

Enantioselectivity of HLADH toward organosilicon compounds was examined by measuring %ee of the remaining alcohols. As illustrated in Fig. 4, enantioselective dehydrogenation of the organosilicon compounds was successfully carried out, especially in the case of **1**. The optical purity of remaining **1** reached higher than 99 %ee at the conversion ratio of 50 %, whereas that of **4** was about 85 %ee, although the optical purity was 99 %ee at 54 % conversion. The optical purity of remaining **3** (34 %ee) was similar to that of **6** (33 %ee) at 50 % conversion. As the conversion ratio increased, however, the difference between the enantioselectivity of HLADH for these two substrates became apparent. At 70 % conversion, for example, **3** showed 70 %ee, while the optical purity of **6** was only 58 %ee. The results shown in Table 1 clearly indicated that the silicon substitution for the carbon atom improved the enantioselectivity of HLADH. The absolute configurations of remaining (-)-**1** and (-)-**4** after the dehydrogenation reaction by HLADH were both *R*, indicating that HLADH was active on the (*S*)-enantiomers of **1** and **4**. Among the substrates investigated, **1** was found to be the most excellent substrate for HLADH on the enantioselective dehydrogenation

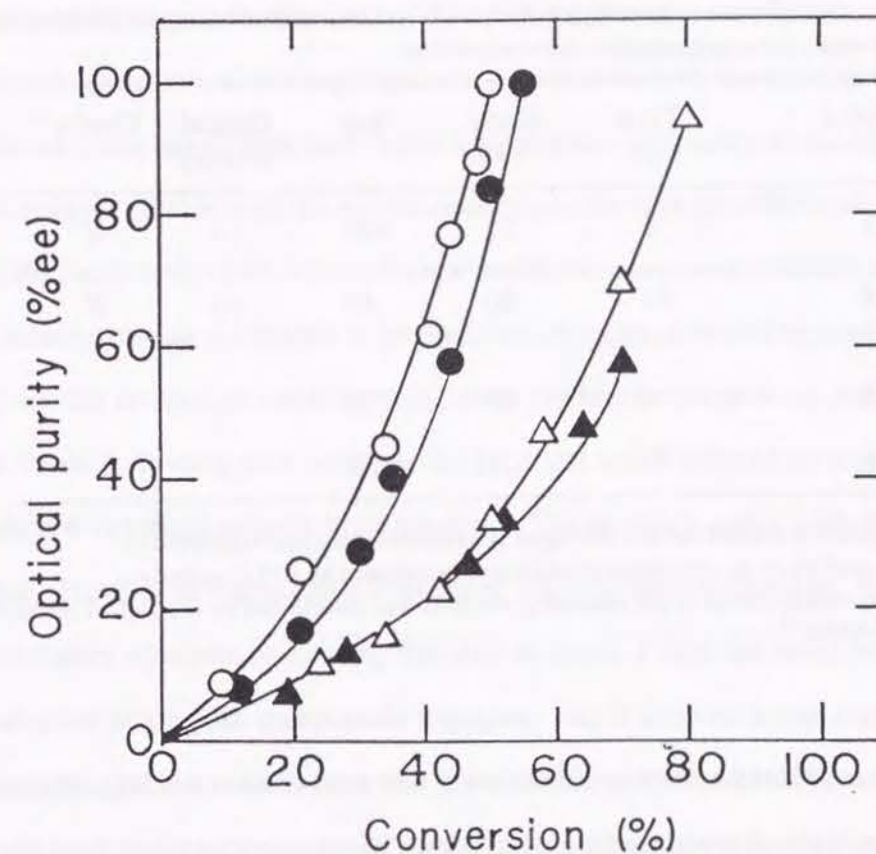


Fig. 4. Relationship between optical purity of the remaining alcohols and conversion ratio on HLADH-catalyzed dehydrogenation in a two-layer system with coenzyme regeneration. Symbols: (○), **1**; (△), **3**; (●), **4**; (▲), **6**.

Table 1. Optically active trimethylsilylpropanols and their carbon analogues obtained by HLADH-catalyzed enantioselective dehydrogenation

Alcohol	Time (h)	Conv. (%)	%ee	Optical activity <sup>a</sup>	Config. <sup>b</sup>
<b>1</b>	9	51	>99	(-)	<i>R</i>
<b>4</b>	41	50	85	(-)	<i>R</i>
<b>3</b>	4	68	70	(-)	
<b>6</b>	9	71	59	(-)	

The reaction was carried out in a two-layer system with coenzyme regeneration.

<sup>a</sup> Optical activity of the remaining alcohols was determined by HPLC analysis.

<sup>b</sup> Absolute configuration of the remaining alcohols was determined by correlated method with <sup>1</sup>H-NMR.<sup>10)</sup>

under the conditions employed because it was converted at the highest rate with the highest enantioselectivity. These phenomena indicate that the substitution of the silicon atom for the carbon atom in substrates breaks through the conventional problem that the more reactive a substrate is, the lower is the stereoselectivity of enzymes.

#### Kinetic analysis in an aqueous monolayer system

The reaction rate of HLADH-catalyzed dehydrogenation in the water-*n*-hexane two-layer system can not be discussed thoroughly without the

information on the diffusion of substrates and products and that on the coenzyme regeneration rate. Therefore, kinetic analysis of the dehydrogenation of the organosilicon compounds and the corresponding carbon compounds was performed spectrophotometrically in an aqueous monolayer system with the excess coenzyme. Because the difference in the enantioselectivity of HLADH between the silicon compounds and their carbon counterparts was not drastic at low conversion ratios, it would be reasonable to use the racemic alcohols for analysis of the kinetic parameters. As shown in Table 2, **4** was a poor substrate for HLADH and **5** showed no reactivity, while **6** exhibited a fairly high reactivity. These results agree with the fact that HLADH is essentially a primary alcohol dehydrogenase. For the secondary alcohols containing the silicon atom, **1** was the most reactive substrate among six compounds examined and **2** showed a low reactivity though its carbon counterparts was not a substrate of HLADH. These results indicate that the replacement of the carbon atom with the silicon atom in the secondary alcohols, **4** and **5**, improved the reactivity of the substrates. In the case of the primary alcohol, however, the reactivity of the silicon substrate **3** was similar to that of its carbon analogue **6**. It is apparent, from the value of *K<sub>m</sub>*, that the silicon substitution for the carbon atom resulted in a higher affinity of the substrates toward HLADH.



Table 2. Kinetic parameters of HLADH-catalyzed dehydrogenation reaction

Alcohol	K <sub>m</sub> (mM)	V <sub>max</sub> ( $\mu\text{mol}\cdot\text{min}^{-1}$ )	V <sub>max</sub> /K <sub>m</sub> ( $10^{-4}\text{ min}^{-1}$ )
<b>1</b>	2.29	0.488	2.13
<b>4</b>	15.7	0.461	1.58
<b>2</b>	7.68	0.137	0.035
<b>5</b>	-	-	-
<b>3</b>	4.62	0.329	0.712
<b>6</b>	7.45	0.462	0.620

Kinetic parameters were obtained in an aqueous monolayer system.

## DISCUSSION

The author has described that the silicon atom increased the reactivity of a substrate having a hydroxyl group on the  $\beta$ -carbon atom but decreased that of a substrate having a hydroxyl group on the  $\alpha$ -carbon atom in HLADH-catalyzed dehydrogenation reaction (PART I, Chapter 1).<sup>9</sup> Furthermore, it was revealed that 2-trimethylsilylethanol, which had a hydroxyl group binding to the  $\beta$ -carbon atom, showed lower activation energy due to the  $\beta$ -effect of the silicon atom<sup>1-2)</sup> and lower frequency factor due to the bulkiness derived

from the longer Si-C bond than its carbon analogue, 3,3-dimethyl-1-butanol. However, the major influence of the activation energy resulted in the higher reactivity of the silicon compound. The fact that **1** has much higher reactivity than its carbon analogue (Table 2) can be well understood, as in the case of 2-trimethylsilylethanol. For **3**, the  $\beta$ -effect of the silicon atom should also enhance the reactivity, but this enhancement seemed not to be great enough to overcome the decrease in reactivity caused by the bulkiness of the trimethylsilyl group, probably because this primary alcohol **3** is more sterically complicated than 2-trimethylsilylethanol. Accordingly, the reactivity of **3** is similar to that of the carbon counterpart. Compound **2** was supposed to be less reactive than its carbon counterpart, as described in PART I, Chapter 1,<sup>9</sup> because its hydroxyl group is bound to the  $\alpha$ -carbon. However, **2** was a better substrate for HLADH than the corresponding carbon compound. This phenomenon was explained by the assumption of the local decrease of steric hindrance derived from the longer Si-C bond. This would make it easy for HLADH to attack the  $\alpha$ -carbon atom in the secondary alcohol. These results revealed that the specific characters of the silicon atom greatly affected the reactivity, though such effect was dependent on the structure of the substrates.

The higher affinity of the silicon compounds toward HLADH, shown as the values of K<sub>m</sub> (Table 2), was attributable to the higher hydrophobicity of the trimethylsilyl group than that of the *tert*-butyl group<sup>13)</sup>. That is, the



trimethylsilyl group is favorable for binding to the hydrophobic active center of HLADH.<sup>14-15)</sup>

In the two-layer system, accumulation of the dehydrogenated products of **4** and **6** was observed, while those of **1** and **3** were not accumulated, because the products,  $\beta$ -carbonylsilanes, were degraded by addition of water into trimethylsilanol and aliphatic carbonyl compounds.<sup>16)</sup> Absence of product inhibition derived from this degradation would be one of the reasons for the higher reaction rates of the silicon compounds observed in the two-layer system. The reason why **2**, which became a substrate for HLADH in the monolayer system, showed no reactivity in the two-layer system is now under exploration.

The effect of the silicon atom on the enantioselectivity of HLADH could be rationally explained based on the structure of the enzyme, that is, the presence of the small and large alkyl-binding pockets in the active site of HLADH.<sup>15)</sup> As a result of the bigger radius of the silicon atom, the large group in **1** is more bulky than that in the corresponding carbon compound and, therefore, more difficult to fit the small alkyl-binding pocket in the active site (Fig. 5A). Consequently, the enantioselectivity of HLADH is enhanced by replacing the carbon atom with the silicon atom. The configuration of remaining **1** after the enantioselective dehydrogenation with HLADH was determined to be *R*, the results being consistent with the Prelog rule<sup>17)</sup> even in the case of the organosilicon compound. Unlike the

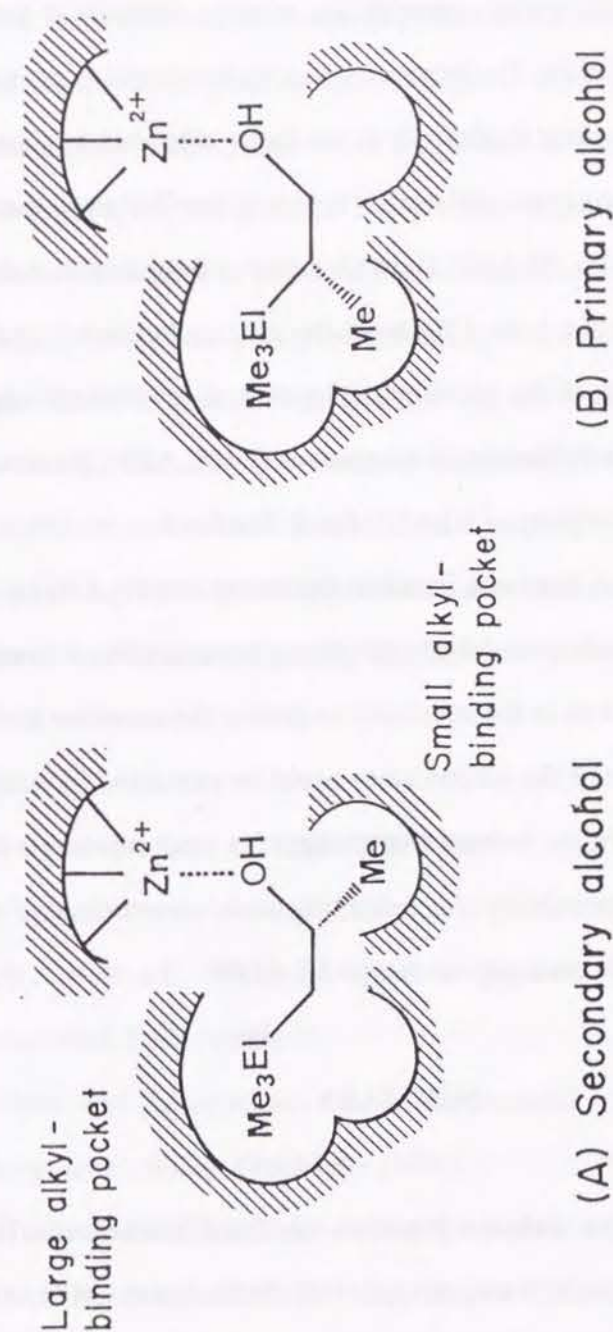


Fig. 5. Hypothetical models of the recognition of enantiomers by HLADH. El=Si or C.

secondary alcohol, the chiral center of the primary alcohols is within the large group of the substrate. The large groups of the two enantiomers supposed to differ from each other in their fit to the large alkyl-binding pocket as shown in Fig. 5B, but this difference is much smaller than that of the secondary alcohol. So HLADH showed rather a poor enantioselectivity toward the primary alcohols. However, the greater bulkiness and/or the larger hydrophobicity of the trimethylsilyl group of **3** would also facilitate the recognition of the difference of enantiomers by HLADH, accounting for the higher enantioselectivity of HLADH for **3** than for **6**.

In conclusion, it has been found in this study that HLADH is able to catalyze the enantioselective dehydrogenation of organosilicon compounds and that the silicon atom in the substrates improves the enantioselectivity of HLADH. The effects of the silicon atom could be explained in terms of its specific characters. To the author's knowledge, this work represents the first demonstration of the possibility of constructing useful stereoselective reaction systems for organosilicon compounds with HLADH.

### SUMMARY

Enantioselective dehydrogenation of three isomers of racemic trimethylsilylpropanols was carried out with horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1.) and the optically active organosilicon

compounds were obtained in a water-organic solvent two-layer system with coenzyme regeneration. Furthermore, the effects of the silicon atom on the enantioselectivity of HLADH were examined in comparison with the corresponding carbon compounds. Substitution of the silicon atom for the carbon atom was found to improve the enantioselectivity of the enzyme. For example, the optical purity of remaining 1-trimethylsilyl-2-propanol was higher than 99 %ee at 50 % conversion, whereas that of the carbon analogue was 85 %ee. This phenomenon was ascribable to the bulkiness of the organosilicon compounds derived from their longer Si-C bond. Kinetic analysis in an aqueous monolayer system demonstrated that the specific properties of the silicon atom greatly affected the reactivity of these substrate compounds.

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### Chapter 3. Enantioselective dehydrogenation of $\beta$ -hydroxysilanes catalyzed by horse liver alcohol dehydrogenase with a novel *in situ* NAD<sup>+</sup> regeneration system

#### INTRODUCTION

As systematic studies on the bioconversion of organosilicon compounds, the author has chosen horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1.) as catalyst, and carried out the dehydrogenation of trimethylsilylalkanols,  $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$  ( $n=1-3$ ) (PART I, Chapter 1),<sup>1)</sup> and three isomers of racemic trimethylsilylpropanols (PART I, Chapter 2).<sup>2)</sup> These studies revealed that the physical properties of the silicon atom greatly affected the activity and enantioselectivity of HLADH toward the unconventional substrates.

NAD(P)(H)-requiring oxidoreductases including HLADH are potentially attractive catalysts in chiral synthesis.<sup>3-5)</sup> However, an efficient cofactor regeneration procedure is indispensable for the practical use of this class of enzymes, because the dissociative cofactors are expensive and unstable in solution. In spite of the developed systems for regeneration of NAD(P)H from NAD(P)<sup>+</sup> in synthetic applications,<sup>6)</sup> it is difficult to establish the efficient reverse reaction, that is, the regeneration of NAD(P)<sup>+</sup> from NAD(P)H, due to thermodynamic disadvantage of enzymatic oxidation of NAD(P)H

and instability of many organic oxidants at a high pH range required for the maximal activity of dehydrogenases (pH~9).<sup>7</sup> Although several regeneration systems have been reported, such as enzyme-catalyzed regeneration,<sup>8</sup> oxidation of the reduced cofactor by dioxygen with electron-transfer reagents,<sup>9</sup> stoichiometric chemical regeneration,<sup>10</sup> electrochemical regeneration,<sup>11</sup> and photocatalytic regeneration,<sup>12</sup> there remains fundamental difficulty for their applications in many dehydrogenase-catalyzed oxidations. For example, product inhibition often provides less efficiency even with an excellent cofactor regeneration system.<sup>7</sup>

This chapter deals with HLADH-catalyzed enantioselective dehydrogenation of  $\beta$ -hydroxysilanes (Fig. 1, 1-5) for preparation of the optically active organosilicon compounds with a novel *in situ* NAD<sup>+</sup> regeneration system based on spontaneous degradation of the dehydrogenated products,  $\beta$ -carbonylsilanes.

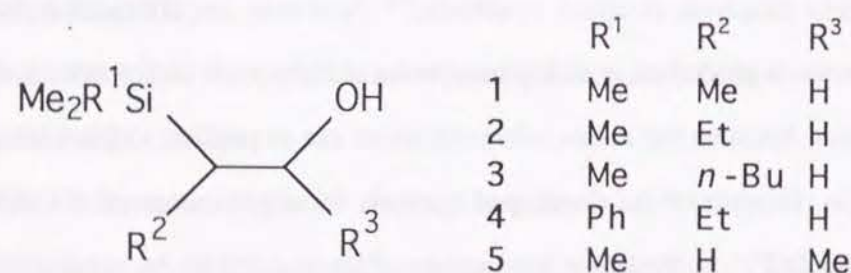


Fig. 1. Structures of racemic  $\beta$ -hydroxysilanes used as substrates for HLADH.

## MATERIALS AND METHODS

### Analyses

<sup>1</sup>H-NMR spectra were measured with a JEOL JNM- $\alpha$ 400 NMR spectrometer and IR spectra with a JASCO IR-810 spectrometer. GLC analyses were carried out using a Shimazu GC-7A equipped with a flame-ionization detector and HPLC analyses were done using a Hitachi L-6000 instrument equipped with an L-4200 UV-Vis detector or a Waters R401 differential refractometer. A Shimazu MPS-200 spectrophotometer was used for rate assay.

### Materials

HLADH was purchased from Sigma, St. Louis, MO, USA.  $\beta$ -Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and its reduced form (NADH) were obtained from Oriental Yeast, Tokyo, Japan. Trimethylsilanol was a product of Shin-Etsu Kagaku, Tokyo, Japan. 2-Trimethylsilyl-1-propanol (1)<sup>13</sup> and 1-trimethylsilyl-2-propanol (5)<sup>14</sup> were donated by Nitto Denko, Osaka, Japan. 2-Trimethylsilyl-1-butanol (2) and 2-dimethylphenylsilyl-1-butanol (4) were prepared as described below. 2-Trimethylsilyl-1-hexanol (3) was prepared as described by Hudrlik *et al.*<sup>15</sup> Preparation of 2,3,3-trimethyl-1-butanol (6) was described in Part I, Chapter 2.<sup>2</sup> Other chemicals were also obtained from commercial sources.



### 2-Trimethylsilyl-1-butanol (2)

Trimethylchlorosilane (4.3 g, 40 mmol) (Shin-Etsu Kagaku) was dropped into a 0.3 l flask containing a mixture of zinc powder (3.3 g, 50 mmol) and ethyl 2-bromobutanoate (9.8 g, 50 mmol) (Nacalai Tesque, Kyoto, Japan) in 100 ml dry benzene under an  $N_2$  atmosphere with stirring. A piece of  $I_2$  was added to initiate the reaction, followed by refluxing for 1 h. The mixture was cooled down to 0 °C and 1 N HCl (100 ml) was added. The organic phase separated was further washed with 1 N HCl and the aqueous phase was extracted with ethyl ether (50 ml x 3). The combined organic phase was dried over  $Na_2SO_4$ , concentrated, and distilled under reduced pressure (13 mmHg). The bp 64 °C fraction was collected to give ethyl 2-trimethylsilylbutanoate as colorless oil (3.5 g, 47 %).

Ethyl 2-trimethylsilylbutanoate obtained above (3.5 g, 19 mmol) was reduced by  $LiAlH_4$  (0.71 g, 19 mmol) in 100 ml dry ethyl ether under an  $N_2$  atmosphere with stirring at room temperature for 1 h. 1 N HCl (100 ml) was added and the organic phase, together with the ethyl ether extract of the aqueous phase, was dried over  $Na_2SO_4$ , concentrated, and distilled under reduced pressure (5 mmHg). The bp 43 °C fraction was collected to give **2** as colorless oil (1.1 g, 41 %):  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  0.01 (s, 9H,  $Si(CH_3)_3$ ), 0.73-0.79 (m, 1H, SiCH), 0.96 (t,  $J=7.5$  Hz, 3H,  $CH_3$ ), 1.44-1.58 (m, 2H,  $CH_2$ ), 3.68 (dd,  $J=7.6$ , 10.7 Hz, 1H,  $CH_2OH$ ), 3.80 (dd,  $J=4.5$ , 10.7 Hz, 1H,  $CH_2OH$ ); IR (neat) 3350, 2950, 2850, 1460, 1380, 1020  $cm^{-1}$ .

### 2-Dimethylphenylsilyl-1-butanol (4)

**4** was prepared from dimethylphenylchlorosilane (Shin-Etsu Kagaku) and 2-bromobutanoate by the same procedure as **2**. Bp 140 °C (22 mmHg);  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  0.31 (s, 6H,  $Si(CH_3)_2$ ), 0.92 (t,  $J=7.5$  Hz, 3H,  $CH_3$ ), 0.98-1.02 (m, 1H, SiCH), 1.42-1.54 (m, 2H,  $CH_2$ ), 3.68 (dd,  $J=6.9$ , 10.7 Hz, 1H,  $CH_2OH$ ), 3.79 (dd,  $J=4.5$ , 10.7 Hz, 1H,  $CH_2OH$ ), 7.33-7.52 (m, 5H,  $SiC_6H_5$ ); IR (neat) 3350, 3060, 2950, 2870, 1420, 1250, 1110, 1020  $cm^{-1}$ .

### *Stoichiometric analysis of HLADH-catalyzed enantioselective dehydrogenation of 1*

( $\pm$ )-**1** (200  $\mu$ mol) was dissolved in 50 mM phosphate buffer (pH 6.9, 20 ml) containing 20 IU HLADH and 5 % tetrahydrofuran (THF). After preincubation for 30 min, 20  $\mu$ mol  $NAD^+$  (1/10 amount toward the substrate) was added to start the reaction and the mixture was incubated at 30 °C with shaking (120 strokes  $\cdot$  min $^{-1}$ ). The concentrations of the substrate and trimethylsilanol were measured with GLC after extraction of the reaction mixture with isopropyl ether. GLC analyses were carried out by using a glass column (diameter 3.0 mm x 1.5 m) packed with PEG-HT supported on Uniport R (GL Sciences, Tokyo, Japan) (carrier gas,  $N_2$ ; flow rate, 60 ml  $\cdot$  min $^{-1}$ ). *n*-Pentadecane was used as the internal standard. HPLC equipped with Wakosil 5C18 column (diameter 4.6 mm x 150 mm) (Wako Jun-yaku,



Osaka, Japan) was used to measure the concentrations of *n*-propanol (mobile phase, water; flow rate, 0.5 ml·min<sup>-1</sup>; detection, refractive index) and *n*-propanal (mobile phase, methanol/water 7:3 (v/v); flow rate, 0.7 ml·min<sup>-1</sup>; detection, UV 254 nm). For *n*-propanal, HPLC analysis was done after derivatization with *O*-*p*-nitrobenzylhydroxylamine hydrochloride<sup>16)</sup> (Nacalai Tesque).

#### *HLADH-catalyzed enantioselective dehydrogenation of $\beta$ -hydroxysilanes with a novel in situ NAD<sup>+</sup> regeneration system*

The reaction was carried out in the phosphate buffer (10 ml) containing racemic substrate (50  $\mu$ mol) shown in Fig. 1, 10 IU HLADH, and 0.5  $\mu$ mol NAD<sup>+</sup> (1/10<sup>2</sup> amount toward the substrate). Other conditions were the same as described above. The reaction mixture was extracted with isopropyl ether, followed by GLC analyses to calculate the conversion ratio. *n*-Pentadecane (for **1**, **2**, and **4**) or *n*-tetradecane (for **3**) was used as the internal standard. The conversion ratio in the dehydrogenation reaction was expressed as follows; the average of the amounts of the substrate consumed and trimethylsilanol formed (for **1**); the average of the amounts of trimethylsilanol and the *n*-alkanol both formed (for **2** and **3**); the amount of the substrate consumed (for **4**). The optical purity of the remaining  $\beta$ -hydroxysilanes was determined with HPLC as the same method as the case of **1** described in PART I, Chapter 2.<sup>2)</sup>

## RESULTS AND DISCUSSION

### *Stoichiometric analysis of HLADH-catalyzed enantioselective dehydrogenation of 1*

When HLADH-catalyzed enantioselective dehydrogenation of **1** was carried out with the use of NAD<sup>+</sup> twice as much as the substrate (42 IU HLADH, 85  $\mu$ mol ( $\pm$ )-**1**, 170  $\mu$ mol NAD<sup>+</sup>, 50 mM Tris-HCl buffer (pH 8.8, 17 ml) containing 5 % THF), the conversion ratio calculated from the increase in absorbance of NADH at 340 nm reached 24.8 % in 60 min. However, the optical purity of remaining (-)-**1** at this time was determined to be 100 %ee by HPLC. This value was over the theoretical maximum calculated from the conversion ratio. That is to say, the conversion obtained from the increase in NADH was not consistent with the true one, suggesting the occurrence of NAD<sup>+</sup> regeneration without any other enzymes and substrates. In addition, the dehydrogenated product, 2-trimethylsilyl-1-propanal (**7**) was never detected by GLC analysis (the similar phenomenon was also seen in PART I, Chapter 2).<sup>2)</sup> But the increase of a different substance, which was identified to be trimethylsilanol, was observed through the reaction. From these results, a novel mode for this enantioselective dehydrogenation was presumed, as shown in Fig. 2. Namely, (+)-**1** is enantioselectively dehydrogenated by HLADH, and (-)-**1** is remained in the reaction mixture. The dehydrogenated product **7**, which is a  $\beta$ -carbonylsilane,



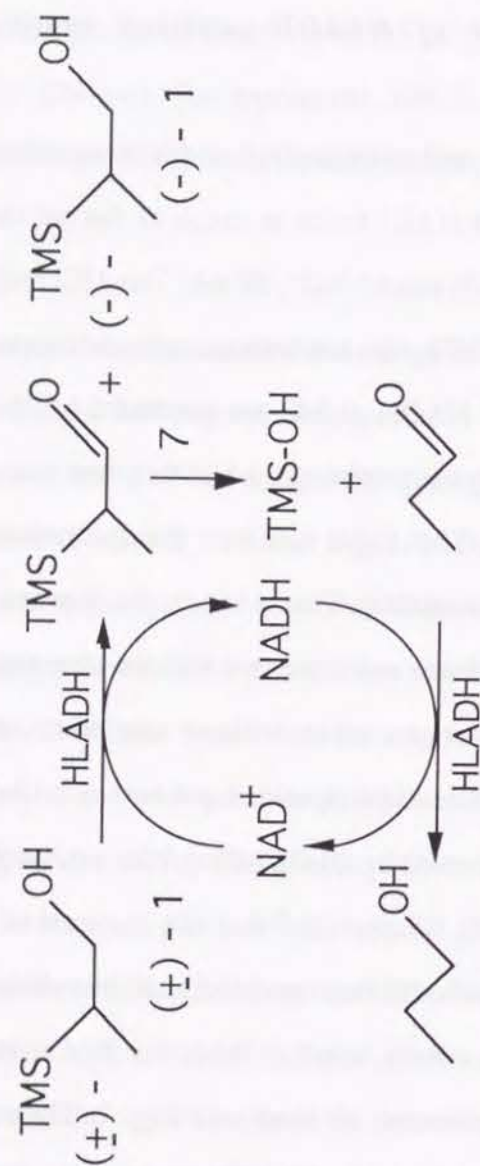


Fig. 2. Reaction scheme of HLADH-catalyzed enantioselective dehydrogenation of 2-trimethylsilyl-1-propanol (1) with *in situ* NAD<sup>+</sup> regeneration. TMS, trimethylsilyl.

is spontaneously degraded by addition of water into trimethylsilanol and *n*-propanal.<sup>17)</sup> As *n*-propanal is acceptable by HLADH as a substrate for the reduction reaction, NAD<sup>+</sup> can be regenerated through the HLADH-catalyzed reduction of *n*-propanal to *n*-propanol. Therefore, the author stoichiometrically examined the reaction with only HLADH and a catalytic amount of NAD<sup>+</sup> to prove this hypothesis by measuring the concentrations of the substances in the reaction mixture with GLC and HPLC.

As illustrated in Fig. 3, the dehydrogenation of 1 catalyzed by HLADH surely proceeded with only a catalytic amount of NAD<sup>+</sup>, judged from the decrease in concentration of the substrate. However, 7 was not detected at all, and trimethylsilanol was formed instead of the organosilicon aldehyde 7 as described above. The amount of trimethylsilanol formed was almost equal to that of the substrate consumed, indicating the quantitative degradation of 7 spontaneously by the addition of water. *n*-Propanal, which was the coproduct of the degradation, existed only a small amount in the mixture through the reaction, whereas its reduced form, *n*-propanol, was detected by HPLC analysis, although the sum of *n*-propanol and *n*-propanal was a little smaller than the amount of trimethylsilanol because of their volatile character. On the whole, the stoichiometric analysis successfully demonstrated that the dehydrogenation reaction with only HLADH and a catalytic amount of NAD<sup>+</sup> proceeded with *in situ* coenzyme regeneration due to the spontaneous degradation of β-carbonylsilane, as shown in Fig. 2.

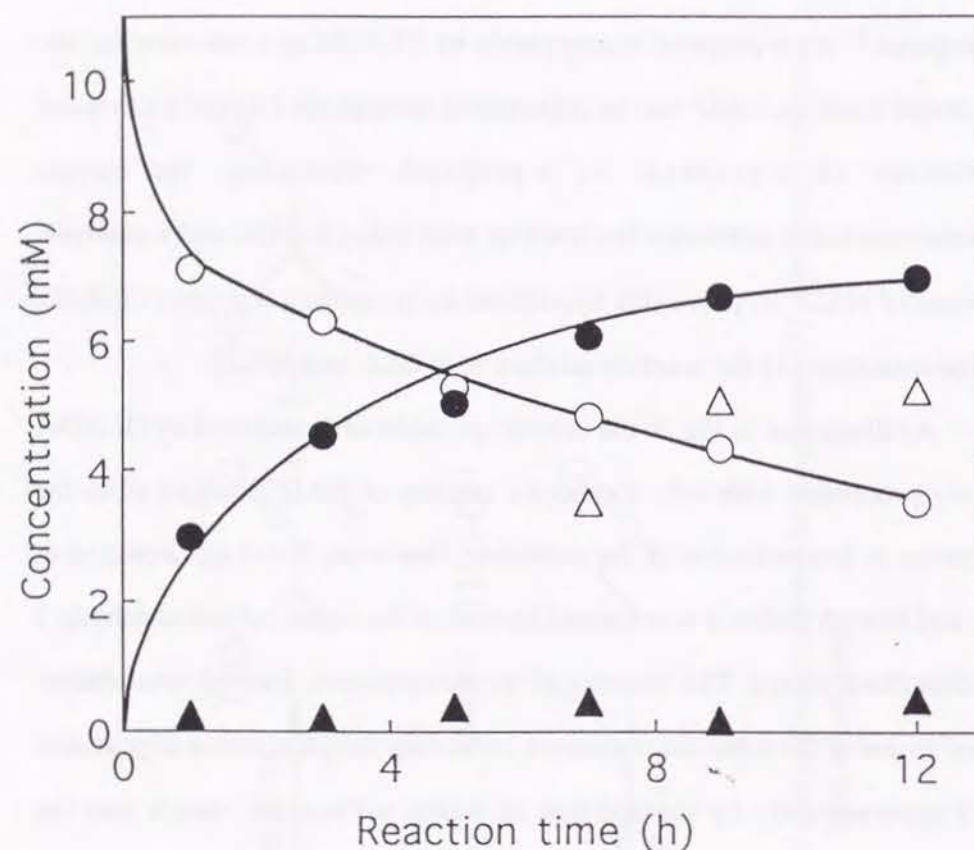


Fig. 3. Time-course of change in concentration of the constituents in HLADH-catalyzed enantioselective dehydrogenation of **1** with *in situ* NAD<sup>+</sup> regeneration. Symbols: (○), **1**; (●), trimethylsilanol; (△), *n*-propanol; (▲), *n*-propanal.

HLADH is known to have different optimum pH for the direction of the oxidation or reduction, that is, pH 8.8 for the dehydrogenation of alcohols with NAD<sup>+</sup> and pH 6.9 for the reduction of carbonyl compounds with NADH. However, the dehydrogenation of **1** with the *in situ* NAD<sup>+</sup> regeneration proceeded higher in phosphate buffer (50 mM, pH 6.9) than in Tris-HCl buffer (50 mM, pH 8.8), because the dehydrogenation of **1** is irreversible due to the spontaneous degradation of the product and the equilibrium of the reduction of *n*-propanal catalyzed by HLADH is shifted toward the desired direction at pH 6.9. Therefore, this slightly acidic condition promoted the *in situ* NAD<sup>+</sup> regeneration shown in Fig. 2 more efficiently than the alkaline condition.

The dehydrogenation of **1** proceeded with a  $1/10^2$  amount of NAD<sup>+</sup> toward the substrate, and 74.5 % conversion was obtained in 14 h (Fig. 4). The optical purity of remaining (-)-**1** increased as the reaction proceeded, as illustrated in Fig. 5. The enantioselectivity of HLADH toward **1** was not complete, as shown by the fact that the optical purity was 69.5 %ee at 58.8 % conversion. However, highly optically pure (-)-**1** (90.1 %ee) was obtained at 74.5 % conversion by this enzyme with the *in situ* NAD<sup>+</sup> regeneration. On the other hand, the dehydrogenation of its carbon counterpart **6** was almost negligible under the same conditions, because the aldehyde formed was not degraded, so that NAD<sup>+</sup> was not regenerated (Fig. 4). **1** was dehydrogenated to give 63.8 % conversion even though the amount of



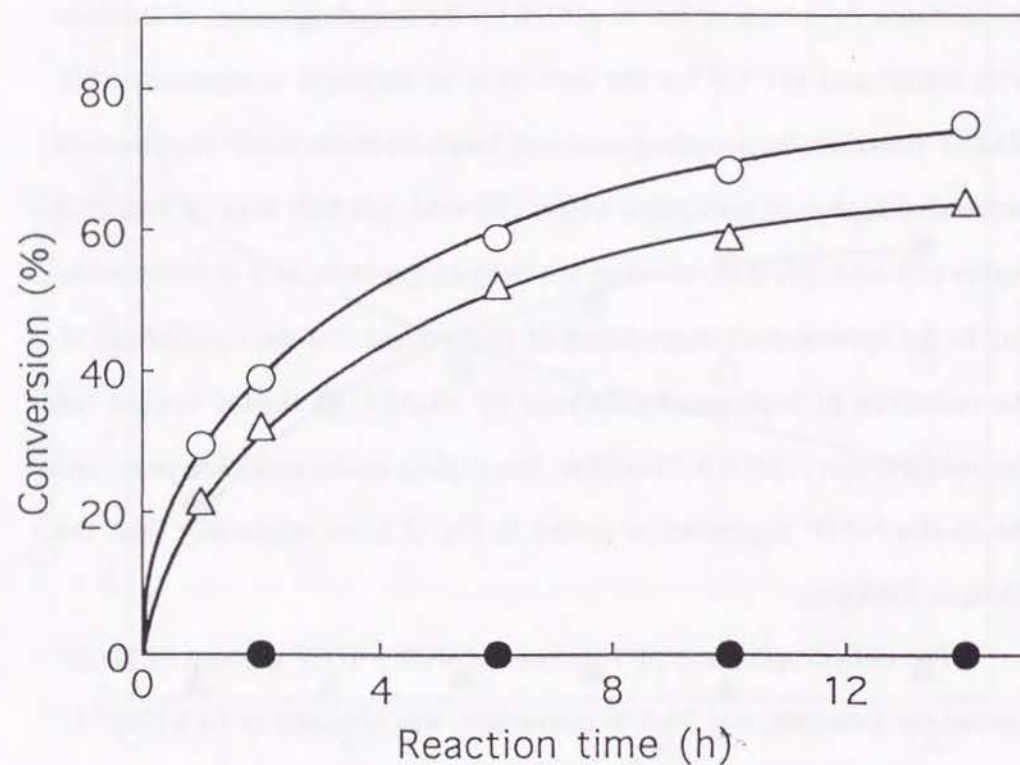


Fig. 4. Time-course of HLADH-catalyzed enantioselective dehydrogenation of **1** and its carbon counterpart **6** with catalytic amounts of  $\text{NAD}^+$ . Symbols: (O), 5.0 mM **1** and  $5.0 \times 10^{-2}$  mM  $\text{NAD}^+$ ; ( $\Delta$ ), 5.0 mM **1** and  $5.0 \times 10^{-3}$  mM  $\text{NAD}^+$ ; ( $\bullet$ ), 5.0 mM **6** and  $5.0 \times 10^{-2}$  mM  $\text{NAD}^+$ .

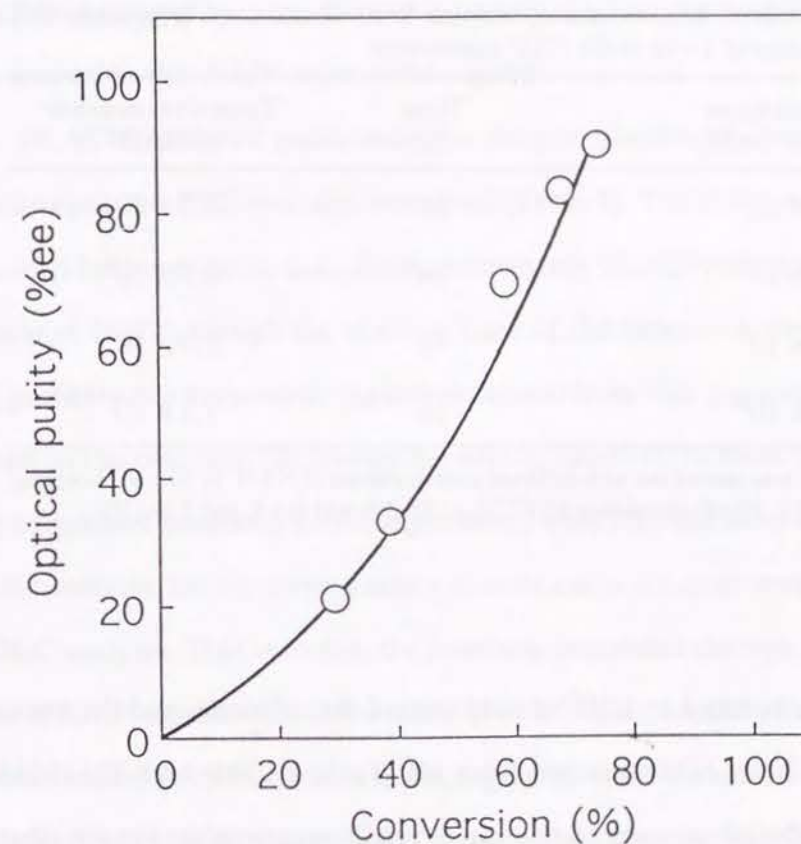


Fig. 5. Relationship between optical purity of remaining (-)-**1** and conversion ratio on HLADH-catalyzed enantioselective dehydrogenation with *in situ*  $\text{NAD}^+$  regeneration.

Table 1. Turnover number of coenzyme in HLADH-catalyzed enantioselective dehydrogenation of **1** with *in situ* NAD<sup>+</sup> regeneration

Concentration of NAD <sup>+</sup> (mM)	Time (h)	Turnover number of NAD <sup>+</sup>
$5.0 \times 10^{-2}$	14	$7.5 \times 10$
$5.0 \times 10^{-3}$	14	$6.5 \times 10^2$
$5.0 \times 10^{-4}$	14	$3.9 \times 10^3$
$5.0 \times 10^{-5}$	14	$7.2 \times 10^3$

The reaction was carried out with different concentrations of NAD<sup>+</sup> in 50 mM phosphate buffer (pH 6.9, 10 ml) containing 10 IU HLADH, 5.0 mM ( $\pm$ )-**1**, and 5 % THF.

NAD<sup>+</sup> was reduced to  $1/10^3$  amount toward the substrate, and the turnover number (TN) of NAD<sup>+</sup> reached  $6.4 \times 10^2$  (Table 1). This high TN of NAD<sup>+</sup> is one of the advantages of this novel NAD<sup>+</sup> regeneration system, that is, there is no need of any other enzymes and substrates for the regeneration and their costs can be omitted. Another advantage is no product inhibition, which is the serious problem in many dehydrogenase-catalyzed oxidations of alcohols, because of the spontaneous degradation of the product. By reducing the concentration of NAD<sup>+</sup> to  $1/10^5$  amount toward the substrate, the reaction rate was decreased, because the number of NAD<sup>+</sup> molecules ( $5.0 \times 10^{-4}$   $\mu$ mol) was smaller than that of the enzyme molecules ( $7.4 \times 10^{-2}$

$\mu$ mol). However, even under these conditions, TN of  $7.2 \times 10^3$  was obtained.

#### *HLADH-catalyzed enantioselective dehydrogenation of $\beta$ -hydroxysilanes with a novel in situ NAD<sup>+</sup> regeneration system*

HLADH-catalyzed enantioselective dehydrogenation of other racemic  $\beta$ -hydroxysilanes (**2-5**) was also examined (Table 2). The reaction with the primary  $\beta$ -hydroxysilanes, **2-4**, proceeded with only HLADH and the catalytic amount of NAD<sup>+</sup>, though the reaction rates of the latter two were not so high probably due to the steric hindrance derived from their large substituent groups on the  $\alpha$ -carbon (**3**) and on the silicon atom (**4**). In these cases, the dehydrogenated products,  $\beta$ -carbonylsilanes, were also not detected in the reaction mixture, but the corresponding silanols and *n*-alkanols were detected by GLC analysis. That is to say, the reactions proceeded through the same mode of NAD<sup>+</sup> regeneration as the reaction of **1**. In contrast, dehydrogenation of the secondary  $\beta$ -hydroxysilane **5** was negligible under the same conditions, although HLADH could convert it with the conventional coenzyme regeneration system (PART I, Chapter 2).<sup>2</sup> The dehydrogenated product of **5**, 1-trimethylsilyl-2-propanone, was confirmed to be degraded by the addition of water into trimethylsilanol and acetone also in this case, but the regeneration of NAD<sup>+</sup> did not occur because acetone was not accepted as the substrate by HLADH and so the dehydrogenation reaction did not proceed over the catalytic amount of NAD<sup>+</sup>.

As shown in Table 2, highly optical pure **2** (97.6 %ee), having a little



Table 2. HLADH-catalyzed enantioselective dehydrogenation of  $\beta$ -hydroxysilanes with *in situ* NAD<sup>+</sup> regeneration

Alcohol	Time (h)	Conv. (%)	%ee <sup>a</sup>	E* <sup>b</sup>
<b>1</b>	6	58.8	69.5	6
<b>2</b>	10	55.0	97.6	39
<b>3</b>	72	22.5	20.0	7
<b>4</b>	96	35.5	52.1	61
<b>5</b>	96	nil		

The reaction was carried out in the phosphate buffer (10 ml) containing 10 IU HLADH, 5.0 mM racemic substrate,  $5.0 \times 10^{-2}$  mM NAD<sup>+</sup>, and 5% THF.

<sup>a</sup> %ee of the remaining  $\beta$ -hydroxysilanes determined by HPLC.

<sup>b</sup>  $E^* = \ln\{(1-c)(1-ee)\} / \ln\{(1-c)(1+ee)\}$ , where *c* is the conversion ratio and *ee* is the enantiomeric excess of the remaining  $\beta$ -hydroxysilanes.<sup>18)</sup>

longer alkyl group ( $R^2=Et$ ) on its  $\alpha$ -carbon atom in comparison with **1**, was successfully prepared by this system at 55.0 % conversion, and E\* value (an indication of enantioselectivity in enzymatic kinetic resolution)<sup>18)</sup> was 39, being much higher than that of **1** ( $E^*=6$ ). However, still longer alkyl group (**3**,  $R^2=n-Bu$ ) reduced the enantioselectivity (20.0 %ee at 22.5 % conversion,  $E^*=7$ ). This phenomenon was well explained based on the importance of enough difference in bulkiness and/or hydrophobicity among the silicon-containing group, the alkyl group, and the hydrogen atom around

the chiral center for enantiomeric recognition with HLADH toward primary  $\beta$ -hydroxysilanes. The greatly increased enantioselectivity of HLADH toward **4** (52.1 %ee at 35.5 % conversion,  $E^*=61$ ) supported this explanation, because its bigger silicon-containing group ( $R^1=Ph$ ) than the trimethylsilyl group caused a quite enough difference among the substituents. The author's previous observation that the silicon substitution for the carbon atom in a primary alcohol increased the enantioselectivity of HLADH (PART I, Chapter 2)<sup>2)</sup> was also consistent with the results obtained here. As it is not well-known how HLADH recognizes the chirality toward primary alcohols compared to that toward secondary alcohols, this information will be useful to discuss the recognition of chirality by HLADH toward primary alcohols even those not containing the silicon atom.

In conclusion, it was proved that NAD<sup>+</sup> could be regenerated in the HLADH-catalyzed dehydrogenation of primary  $\beta$ -hydroxysilanes through reduction of aldehydes formed by spontaneous degradation of the dehydrogenated products,  $\beta$ -carbonylsilanes, and the silicon-containing alcohols were optically resolved by HLADH with the novel *in situ* NAD<sup>+</sup> regeneration system. The author has shown that introduction of organosilicon compounds is not only interesting in basic investigation for enzyme-catalyzed reactions, but also important for production of optically active organosilicons. This study, furthermore, indicates that novel reaction systems will be able to be constructed by applying the specific properties of organosilicon

compounds.

### SUMMARY

Dehydrogenation of 2-trimethylsilyl-1-propanol was carried out with horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1.). It was found that the dehydrogenation proceeded enantioselectively with only HLADH and a catalytic amount of  $\text{NAD}^+$  due to *in situ*  $\text{NAD}^+$  regeneration based on a specific property of  $\beta$ -carbonylsilanes. That is, the (+)-enantiomer was selectively dehydrogenated by HLADH to 2-trimethylsilyl-1-propanal, which was spontaneously degraded by addition of water into trimethylsilanol and *n*-propanal. Then,  $\text{NAD}^+$  was regenerated through HLADH-catalyzed reduction of *n*-propanal to *n*-propanol. On the other hand, dehydrogenation of its carbon analogue was negligible with the catalytic amount of  $\text{NAD}^+$ , indicating that the *in situ*  $\text{NAD}^+$  regeneration was not available without the specific property of organosilicon compounds. Other primary  $\beta$ -hydroxysilanes having different substituents on the chiral center or on the silicon atom were also found to serve as substrates in the enantioselective dehydrogenation by HLADH with this novel  $\text{NAD}^+$  regeneration system. Chiral recognition of HLADH toward primary alcohols was also discussed.

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## PART II Bioconversion of Organosilicon Compounds by Hydrolases

### Chapter 1. Efficient kinetic resolution of trimethylsilylpropanols by enantioselective esterification with hydrolases in organic solvent

#### INTRODUCTION

Enzymes have been utilized as useful catalysts with high selectivity in synthetic chemistry, especially in stereoselective and regioselective synthesis.<sup>1-2)</sup> However, this high selectivity itself prevents the wide application of enzymes.

To increase further the potential of enzymes, the author's laboratory has carried out the bioconversion of non-natural organosilicon compounds, and reported that the silicon atom in substrates served not only as a mimic carbon atom but also as a potent atom to affect enzymatic reactions,<sup>3-6)</sup> due to the lower electronegativity and the bigger atomic radius of the silicon atom. On kinetic resolution of three isomers of racemic trimethylsilylpropanols (Fig.1 1-3) with HLADH, it was revealed that the silicon atom enhanced the enantioselectivity of the enzyme, but efficient resolution of **2** and **3** was not achieved because of the inactivity of HLADH toward **2** and

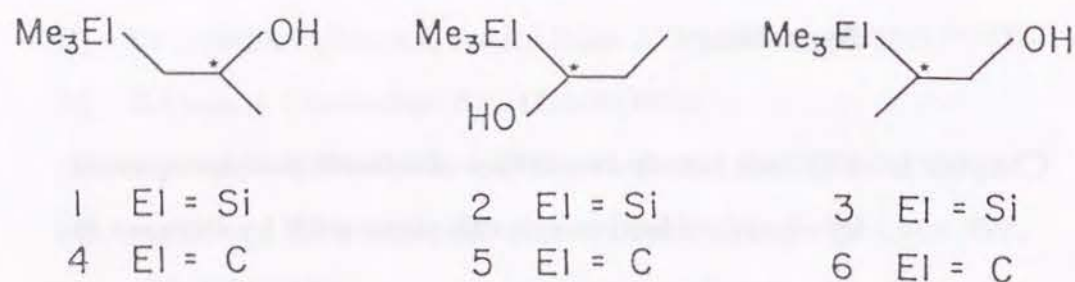


Fig. 1. Structure of three isomers of racemic trimethylsilylpropanols and their carbon analogues used as substrates for hydrolases.

the low enantioselectivity toward **3** (PART I, Chapter 2 and Chapter 3).<sup>5-6)</sup>

In this chapter, the author selected hydrolases as enzymes for the efficient kinetic resolution of the silicon-containing alcohols, because hydrolases are readily available and generally stable, and catalyze the simple reactions without cofactors. Then enantioselective esterification of the racemic trimethylsilylpropanols (**1-3**) by hydrolases was carried out with 5-phenylpentanoic acid as an acyl donor in an organic solvent system. Furthermore, comparative studies were made by using their carbon analogues (Fig. 1 **4-6**) to investigate how the silicon atom affected the hydrolase-catalyzed enantioselective esterification, and the effects observed were discussed based on the properties of the silicon atom.

## MATERIALS AND METHODS

### Analyses

<sup>1</sup>H-NMR and <sup>19</sup>F-NMR spectra were measured with a Varian Gemini-200 NMR spectrometer, and IR spectra with a JASCO IR-810 spectrometer. GLC analyses were carried out using a Shimadzu GC-12A equipped with a flame-ionization detector, and HPLC analyses were done using a Hitachi L-6000 instrument equipped with an L-4200 UV-Vis detector. Specific rotations were determined with a JASCO DIP-140 polarimeter.

### Enzymes

The following five kinds of hydrolase preparations were used: Lipase OF 360 from *Candida cylindracea* (Meito Sangyo, Tokyo, Japan); lipase Saiken 100 from *Rhizopus japonicus* (Osaka Saikin Kenkyusho, Osaka, Japan); lipase (Steapsin) from hog pancreas (Tokyo Kasei, Tokyo, Japan); lipoprotein lipase Type A from *Pseudomonas* sp. (Toyobo, Osaka, Japan); and cholesterol esterase Type A from *Pseudomonas* sp. (Toyobo).

### Chemicals

1-Trimethylsilyl-2-propanol (**1**),<sup>7)</sup> 1-trimethylsilyl-1-propanol (**2**),<sup>8)</sup> and 2-trimethylsilyl-1-propanol (**3**)<sup>8)</sup> were donated by Nitto Denko, Osaka, Japan. 4,4-Dimethyl-2-pentanol (**4**), 2,2-dimethyl-3-pentanol (**5**), and 5-phenyl-



pentanoic acid were purchased from Aldrich, Milwaukee, WI, USA. Preparation of 2,3,3-trimethyl-1-butanol (**6**) was described in PART I, Chapter 2.<sup>9</sup> Celite No.535 was a product of Johns-Manville, Denver, CO, USA. (*R*)-methoxytrifluoromethylphenylacetic acid (MTPA) was purchased from Nacalai Tesque, Kyoto, Japan. All other chemicals were also obtained from commercial sources.

#### *Adsorption of enzyme on Celite*

Enzyme preparation (100 mg) suspended in 100  $\mu$ l deionized water was mixed thoroughly with 250 mg Celite No.535.

#### *Enzymatic reaction*

The reaction mixture was composed of Celite-adsorbed hydrolase (corresponding to 100 mg enzyme) and 10 ml water-saturated 2,2,4-trimethylpentane containing 100 mM racemic alcohol shown in Fig. 1 and 100 mM 5-phenylpentanoic acid. The reaction was carried out at 30 °C with shaking (120 strokes·min<sup>-1</sup>). The ester formed was determined by GLC using a glass column (diameter 3.0 mm  $\times$  1.0 m) packed with PEG-HT supported on Uniport R (GL Sciences, Tokyo, Japan) (carrier gas, N<sub>2</sub>; flow rate, 60 ml·min<sup>-1</sup>) using *n*-pentadecane as the internal standard. The optical purity of the remaining alcohols after the esterification with hydrolases was determined with HPLC (PART I, Chapter 2),<sup>9</sup> or <sup>1</sup>H- and <sup>19</sup>F-NMR after

derivatization with (*R*)-MTPA.<sup>9</sup> Specific rotation of the remaining alcohols was measured in ethanol after purification by column chromatography on Silica gel 60 (No.7734, Merck, Darmstadt, FRG) (mobile phase; *n*-pentane/ethyl ether, 10:1 v/v for the ester and 4:1 v/v for the alcohol).

#### *Determination of absolute configuration*

The absolute configuration of **2** was determined by the correlation method with <sup>1</sup>H-NMR.<sup>10</sup> Diastereomeric esters of (-)-**2** obtained and its racemic compound were prepared with (*R*)-MTPA and analyzed. The signals of each enantiomer were identified as follows.

[(*R*)-MTPA] ester of (*R*)-(-)-**2**. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  -0.038 (s, 9H, Si(CH<sub>3</sub>)<sub>3</sub>), 0.92 (t, *J*=7.4 Hz, 3H, CH<sub>3</sub>), 1.6-1.8 (m, 2H, CH<sub>2</sub>), 3.54 (q, *J*=1.3 Hz, 3H, OCH<sub>3</sub>), 4.91 (t, *J*=7.1 Hz, 1H, CH), 7.4-7.6 (m, 5H, C<sub>6</sub>H<sub>5</sub>).

[(*R*)-MTPA] ester of (*S*)-(+)-**2**. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  0.032 (s, 9H, Si(CH<sub>3</sub>)<sub>3</sub>), 0.85 (t, *J*=7.3 Hz, 3H, CH<sub>3</sub>), 1.6-1.8 (m, 2H, CH<sub>2</sub>), 3.52 (q, *J*=1.1 Hz, 3H, OCH<sub>3</sub>), 4.91 (t, *J*=6.9 Hz, 1H, CH), 7.4-7.6 (m, 5H, C<sub>6</sub>H<sub>5</sub>).

The configurations of **1** and **4** were determined as described in PART I, Chapter 2.<sup>9</sup>

## RESULTS

Time-course of enantioselective esterification of three isomers of

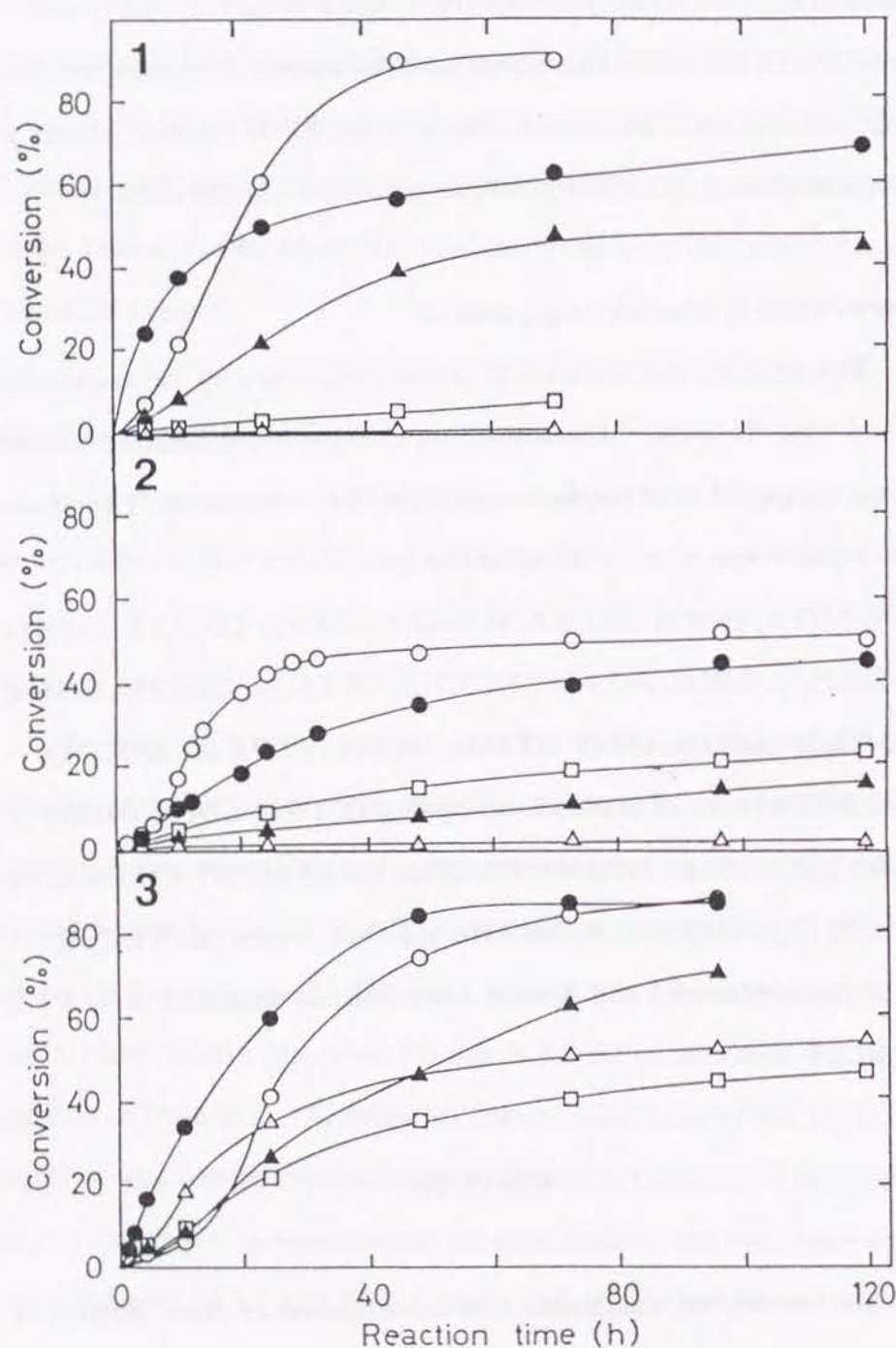


Fig. 2. Time-course of hydrolase-catalyzed esterification of the organosilicon compounds with 5-phenylpentanoic acid in water-saturated 2,2,4-trimethylpentane. The numbers in the each panels indicate the substrate used. Symbols: (○), Lipase OF 360; (△), lipase Saiken 100; (□), lipase (Steapsin); (●), lipoprotein lipase Type A; (▲), cholesterol esterase Type A.

racemic trimethylsilylpropanols (**1-3**) and their carbon counterparts (**4-6**) with 5-phenylpentanoic acid catalyzed by five kinds of hydrolases is shown in Fig.2 and Fig.3, respectively. The initial reaction rates and the optical purity of the remaining alcohols at the conversion ratio of about 50 % are summarized in Table 1. Of five kinds of hydrolases used, lipase OF 360 and lipoprotein lipase were active toward all the alcohols examined except for **5**, whereas lipase Saiken 100 was active only toward the primary alcohols. The highly optically active silicon-containing alcohols could be prepared by this enzymatic method, that is, 93 %ee of **1** with cholesterol esterase, 96 %ee of **2** with lipoprotein lipase, and 95 %ee of **3** with lipase Saiken 100 (Table 1).

It is worth noting that the  $\beta$ -hydroxysilanes, **1** and **3**, were effectively esterified with enantioselectivity by the hydrolases in organic solvent, because they were easily converted to alkenes *via*  $\beta$ -elimination under both acidic and basic conditions (Peterson olefination)<sup>11)</sup> and therefore, it is not possible to esterify them by chemical catalysts like acids. The enzymes could convert



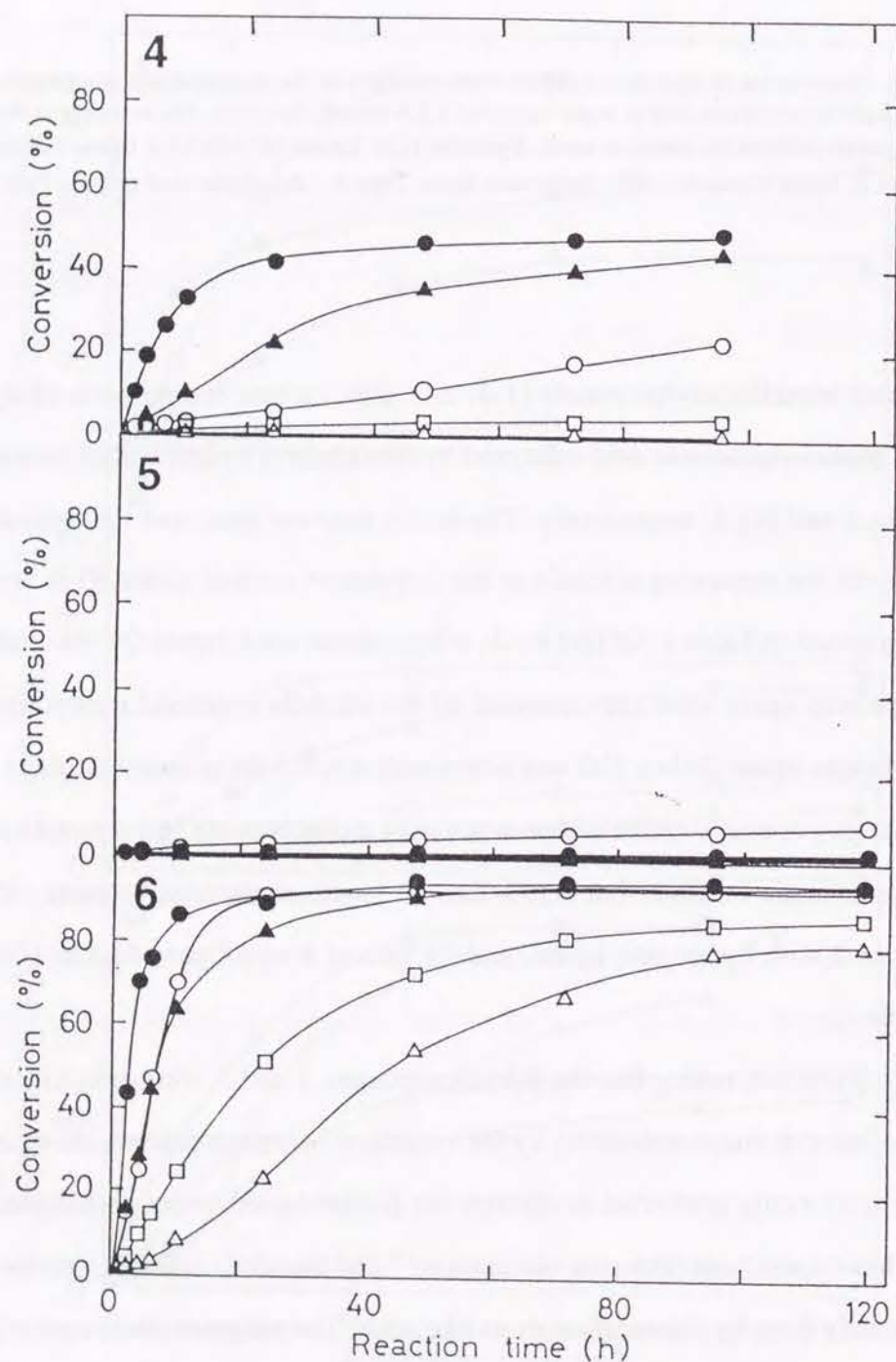


Fig. 3. Time-course of hydrolase-catalyzed esterification of the corresponding carbon compounds with 5-phenylpentanoic acid in water-saturated 2,2,4-trimethylpentane. The numbers in the each panels indicate the substrate used. Symbols: (○), Lipase OF 360; (△), lipase Saiken 100; (□), lipase (Steapsin); (●), lipoprotein lipase Type A; (▲), cholesterol esterase Type A.

such unstable compounds with recognizing their chirality under the mild conditions. This fact is one example showing the effectiveness of introduction of biochemical methods into organosilicon chemistry.

When the silicon compounds are compared with their carbon counterparts, the secondary alcohol **1** was as reactive as **4** for the hydrolases except for lipase OF 360. However, the optical purity of remaining **1** in the case of lipoprotein lipase and cholesterol esterase (91 and 93 %ee, respectively) was higher than that of remaining **4** (86 and 88 %ee, respectively), although the difference was not so large. It is very interesting that lipase OF 360 and lipoprotein lipase also esterified the more complicated secondary alcohol **2** with high enantioselectivity, while its carbon analogue **5** was hardly esterified. The enantioselectivity of lipase OF 360 and lipoprotein lipase toward **2** was higher than that toward **1**, probably because of the steric effect around the hydroxyl group. The primary alcohol **3** was a less reactive substrate than the carbon analogue **6** for these hydrolases except for lipase Saiken 100, but the hydrolases except for lipoprotein lipase showed

Table 1. Initial reaction rate and optical purity of remaining alcohols on hydrolase-catalyzed enantioselective esterification

Alcohol	Lipase OF 360		Lipase Saiken 100		Lipase (Steapsin)		Lipoprotein lipase		Cholesterol esterase	
	Rate <sup>a</sup>	%ee <sup>b</sup> (Conv. %)	Rate	%ee (Conv. %)	Rate	%ee (Conv. %)	Rate	%ee (Conv. %)	Rate	%ee (Conv. %)
<b>1</b>	0.21	8 (49)	0.00		0.01		0.37	91 <sup>c</sup> (48)	0.08	93 <sup>c</sup> (49)
<b>4</b>	0.03	15 (62)	0.00		0.01		0.31	86 (49)	0.10	88 (50)
<b>2</b>	0.18	89 (48)	0.02		0.05		0.10	96 (49)	0.04	
<b>5</b>	0.02		0.00		0.01		0.01		0.00	
<b>3</b>	0.17	17 (50)	0.18	95 (50)	0.09	61 (50)	0.34	0 <sup>d</sup> (55)	0.10	48 (49)
<b>6</b>	0.70	7 (48)	0.08	45 (49)	0.24	23 (49)	0.86	18 (51)	0.64	33 (51)

The reaction was carried out with 5-phenylpentanoic acid and 100 mg hydrolase adsorbed on Celite in water-saturated 2,2,4-trimethylpentane.

<sup>a</sup> Initial ester formation rate ( $\mu\text{mol} \cdot \text{h}^{-1} \cdot (\text{mg enzyme})^{-1}$ ).

<sup>b</sup> Optical purity of the remaining alcohols determined by HPLC.

<sup>c</sup> Determined by  $^{19}\text{F}$ -NMR after derivatization with (*R*)-MTPA.<sup>9</sup>

<sup>d</sup> Determined by  $^1\text{H}$ -NMR after derivatization with (*R*)-MTPA.<sup>9</sup>

a higher enantioselectivity on **3** than on **6**. Lipase Saiken 100 showed not only a higher activity but also a much higher enantioselectivity toward **3**. The optical purity of remaining **3** with this enzyme reached 95 %ee, while that of **6** was only 45 %ee. Highly optically active **3** could be obtained in spite of the fact that it is generally difficult to esterify primary alcohols with high enantioselectivity. In this limited investigation, the enantioselectivity of the enzymes toward the silicon-containing alcohols tends to be similar or higher compared to that toward the carbon analogues, and, especially in a certain combination of the substrate and enzyme, the enantioselectivity is drastically enhanced by the silicon substitution. Furthermore, the effect of the silicon atom for the reactivity of the substrates is also dependent greatly on their structure. Steric hindrance around the hydroxyl group, in general, decreases the reactivity. This tendency was clearly seen among the carbon compounds, whereas the silicon substitution for the carbon atom diminished in part such the difference in reactivity.

## DISCUSSION

Previously, the author's laboratory has reported that the specific characters of the silicon atom played important roles in enzymatic reactions.<sup>3-6</sup> For example, trimethylsilylmethanol was an excellent acyl acceptor than its carbon analogue on lipase-catalyzed esterification, probably due to the higher



nucleophilicity of the hydroxyl oxygen atom derived from the lower electronegativity of the silicon atom and the smaller steric hindrance around the hydroxyl group derived from the longer Si-C bond length.<sup>3)</sup>

Compound **2**, which is an  $\alpha$ -hydroxysilane similar to trimethylsilyl-methanol, was a better substrate for the hydrolases than **5**. As discussed above, the higher nucleophilicity of the hydroxyl oxygen atom and the smaller steric hindrance around the hydroxyl group derived from the properties of the silicon atom result in the higher reactivity of **2**, although the more bulky trimethylsilyl group seems to be disadvantageous to the reaction.

Both **1** and **3** are  $\beta$ -hydroxysilanes. On the whole, **1** (secondary alcohol) was esterified with the hydrolases at nearly the same rate as its carbon counterpart, and **3** (primary alcohol) was less reactive than its analogue. The effect of the silicon atom shown in the reaction of **2** will be weaker in the  $\beta$ -hydroxysilanes because the hydroxyl group is far from the silicon atom. Therefore, the disadvantage to reactivity caused by the more bulky trimethylsilyl group would offset the favorable effect of the silicon atom, especially in the case of **3**.

The hydrolases generally showed a higher enantioselectivity toward the silicon compounds than toward the corresponding carbon compounds (Table 1). The more bulky trimethylsilyl group supposed to be favorable for the enantiomeric recognition of enzymes. Lipoprotein lipase and cholesterol esterase exhibited a slightly higher enantioselectivity toward **1** (93 and 91

Table 2. Optically active trimethylsilylpropanols and their carbon analogues obtained by hydrolase-catalyzed enantioselective esterification

Alcohol	Hydrolase used	%ee <sup>a</sup>	Config. <sup>b</sup>	$[\alpha]_D^c$
<b>1</b>	Lipase OF 360	8	<i>R</i>	+4.2
	Lipoprotein lipase	91	<i>S</i>	
	Cholesterol esterase	93	<i>S</i>	
<b>4</b>	Lipase OF 360	15	<i>S</i>	+26.8
	Lipoprotein lipase	86	<i>S</i>	
	Cholesterol esterase	88	<i>S</i>	
<b>2</b>	Lipase OF 360	89	<i>R</i> <sup>d</sup>	-5.8
	Lipoprotein lipase	96	<i>R</i> <sup>d</sup>	
<b>3</b> <sup>e</sup>	Lipase OF 360	17		-5.3
	Lipase Saiken 100	95		
	Lipase (Steapsin)	61		
<b>6</b> <sup>e</sup>	Lipase OF 360	7		-19.0
	Lipase Saiken 100	45		
	Lipase (Steapsin)	23		

The reaction was carried out with 5-phenylpentanoic acid and 100 mg hydrolase adsorbed on Celite in water-saturated 2,2,4-trimethylpentane.

<sup>a</sup> See, Table 1.

<sup>b</sup> Absolute configuration of the remaining alcohols after the esterification was determined by correlation method with <sup>1</sup>H-NMR.<sup>10)</sup>

<sup>c</sup> Specific rotation was measured at 1 % w/v in ethanol at room temperature except for the case of **6** (0.3 % w/v).

<sup>d</sup> (-)-(*R*)-**2** has the same relative configuration as (*S*)-**1** or (*S*)-**4**, although the designation of *RS* is opposite due to the sequence rule.

<sup>e</sup> The remaining alcohols obtained were the (-)-enantiomers except for lipase OF 360.



%ee of the remaining alcohol) than toward **4** (88 and 86 %ee), and lipoprotein lipase converted also **2** with high enantioselectivity (96 %ee). These enzymes recognized the (*R*)-enantiomers of **1** and **4**, and the (*S*)-enantiomer of **2** as substrates (Table 2). Lipase OF 360 was a unique enzyme because the reaction rate of **1** was much higher than that of **4** and an inversion of the enantioselectivity from *R* to *S* was observed by the substitution of the silicon atom for the carbon atom, although the optical purity attained were not so high. While Syldatk *et al.*<sup>12)</sup> reported that microbial reduction of acetyl-dimethylphenylsilane and their carbon and germanium analogues resulted in (*R*)-selectivity regardless of the kind of center atom (C, Si, and Ge), the author observed the inversion of enantioselectivity depending on the kind of center atom, and such inversion has not been reported previously. Furthermore, highly optically active **3** (95 %ee) could be obtained by using lipase Saiken 100. Generally speaking, it is difficult to carry out the highly stereoselective conversion of compounds having functional groups far from the chiral center, such as primary alcohols, both with enzymatic and chemical methods. The author's laboratory examined 50 kinds of hydrolases for optical resolution of citronellol by enantioselective esterification in an organic solvent system, but no enzyme showed the selectivity toward the primary terpene alcohol.<sup>13)</sup> The optical purity of **6** obtained was also not so high. However, effective resolution of the primary alcohol **3** was achieved by introducing the silicon atom into the structure and by using lipase Saiken 100.

Kinetic resolution of these organosilicon compounds was also tried by HLADH-catalyzed enantioselective dehydrogenation with coenzyme regeneration (PART I, Chapter 2 and Chapter 3).<sup>5-6)</sup> However, the dehydrogenase showed no activity toward **2** and a low enantioselectivity toward **3**. The use of hydrolases enabled the efficient kinetic resolution of all the organosilicon compounds examined without any cofactors.

In conclusion, the author has found that the hydrolases can effectively catalyze the enantioselective esterification of silicon-containing alcohols, even  $\beta$ -hydroxysilanes which are unstable under the conditions of acid-catalyzed esterification, with 5-phenylpentanoic acid in an organic solvent system. The highly optically active trimethylsilylpropanols, including a primary alcohol, could be obtained with adequate hydrolases. Furthermore, it was revealed that the silicon atom often enabled enhancement of the enantioselectivity of the enzymes and affected the reactivity of the substrates in a different manner depending on their structure.

## SUMMARY

Enantioselective esterification of three isomers of racemic trimethylsilylpropanols, 1-trimethylsilyl-2-propanol, 1-trimethylsilyl-1-propanol, and 2-trimethylsilyl-1-propanol, was systematically studied with five kinds of hydrolases in an organic solvent system in connection with the



structure of the compounds. The hydrolases were found to be able to esterify these organosilicon compounds, even  $\beta$ -hydroxysilanes which are unstable under the conditions of acid-catalyzed esterification, and the highly optically active organosilicon compounds were successfully prepared with the selected hydrolases. Even a primary alcohol, 2-trimethylsilyl-1-propanol, was enantioselectively esterified by lipase. Furthermore, comparative studies were made by using their carbon counterparts. The silicon atom in the substrates was found to often enhance the enantioselectivity of the enzymes, but its effect on the reactivity of the substrates was dependent on their structure. These results were discussed based on the specific characters of the silicon atom.

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## Chapter 2. Chemoenzymatic preparation of optically active silylmethanol derivatives having an asymmetric silicon atom by hydrolase-catalyzed enantioselective esterification

### INTRODUCTION

Silicon is the second most abundant element in the Earth's crust, and it is always found in combination with oxygen as silica or metal silicates. Although silicon belongs to the same group as carbon in the periodic table and has some properties similar to carbon, such as making tetrahedral  $sp^3$ -hybridized bonds, organosilicon compounds possessing silicon-carbon bonds have never been known to occur in nature. The organosilicon compounds show unique chemical and physical properties compared to conventional organic compounds due to the specific characteristics of silicon, so they are used as important reagents in synthetic chemistry and chemical industry.<sup>1-2)</sup>

The author's laboratory has examined to introduce such organosilicon compounds into bioconversion systems as unconventional substrates,<sup>3-7)</sup> and the author carried out enantioselective conversion of racemic trimethylsilylpropanols with alcohol dehydrogenase (PART I, Chapter 2 and Chapter 3)<sup>5-6)</sup> and hydrolases (PART II, Chapter 1).<sup>7)</sup> The results demonstrated promising methods for preparation of optically active organosilicon

compounds by utilizing the selectivity of biocatalysts.

In this chapter, preparation of optically active silanes having an asymmetric silicon atom with enzymes was attempted. This try is important because recent interest is focused on the use of such chiral silanes<sup>8-9)</sup> as new synthetic reagents,<sup>10-11)</sup> biologically active compounds,<sup>12-15)</sup> and their precursors. It was reported that silicon-analogues of antimuscarinic drugs having a chiral silanol structure were found to exhibit higher pharmacological activity than their corresponding carbon compounds, and that their enantiomers showed a stereoselectivity of antimuscarinic action *in vitro*.<sup>13-14)</sup>

The author selected ethylmethylphenylsilylmethanol (**1**) and its derivatives (**2-9**), which are primary alcohols having an asymmetric silicon atom, as substrates for enzymes (Fig. 1) and tried their kinetic resolution by enantioselective esterification and transesterification with hydrolases in an organic solvent system (Fig. 2). It is interesting to see whether enzymes can recognize the chirality on the silicon atom or not, and to investigate the behavior of enzymes toward these organosilicon compounds. Furthermore, chemical synthesis of chiral quaternary silanes having no leaving groups attached to the silicon atom with high optical purity is generally difficult, even though several methods have been developed for preparing optically active organosilicons. So the use of biocatalysts will provide a new useful procedure to organosilicon chemistry for the preparation of optically active silanes.



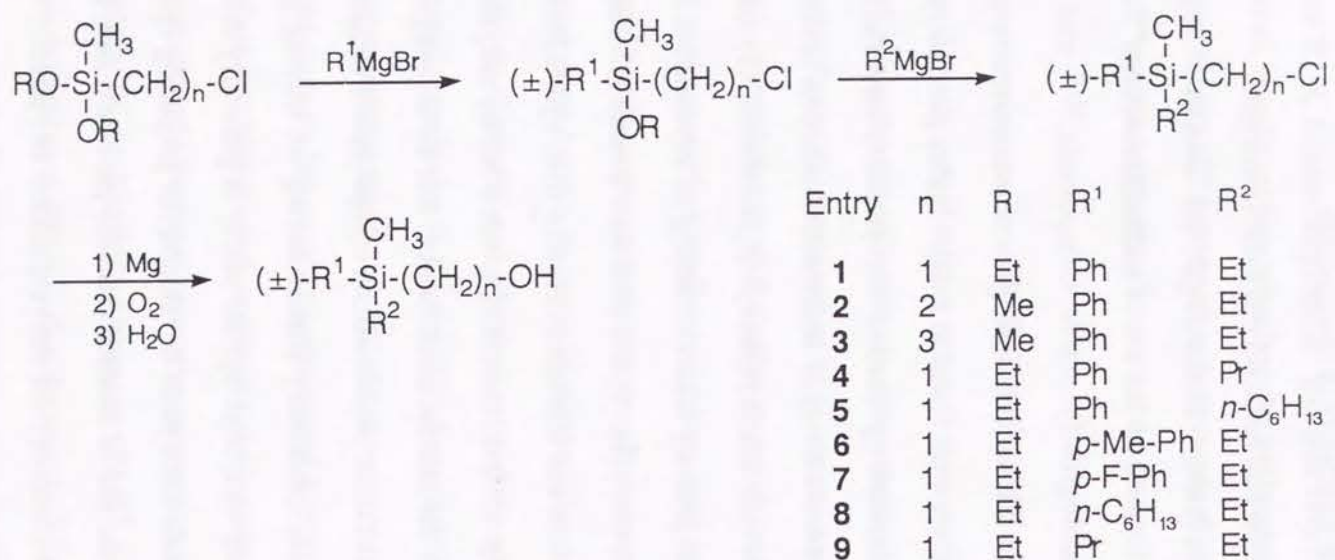


Fig. 1. Preparation of racemic alcohols having an asymmetric silicon atom.

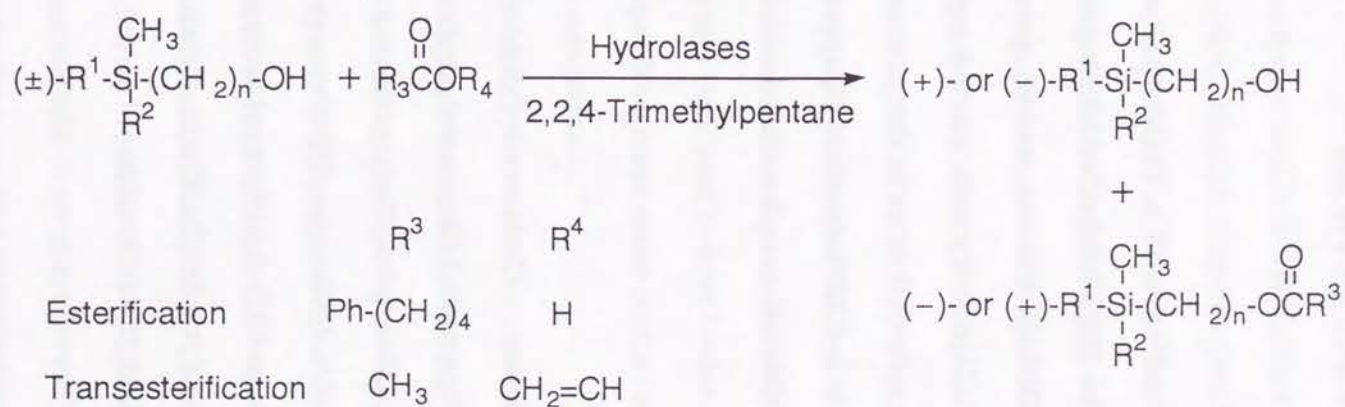


Fig. 2. Enantioselective esterification and transesterification of racemic alcohols having an asymmetric silicon atom catalyzed by hydrolases in an organic solvent system.

## MATERIALS AND METHODS

### Analyses

<sup>1</sup>H-NMR spectra were measured with a JEOL PMX-60 NMR spectrometer, <sup>19</sup>F-NMR spectra with a JEOL JNM-A500 NMR spectrometer, EI mass spectra with a JEOL DX-300 spectrometer, and IR spectra with a JASCO IR-810 spectrometer. GLC analyses were carried out using a Shimadzu GC-14A equipped with a flame-ionization detector, and HPLC analyses were done using a Hitachi L-6000 instrument equipped with an L-4200 UV-Vis detector. Specific rotations were determined with a JASCO DIP-140 polarimeter.

### Chemicals

Chloromethyldiethoxymethylsilane, (2-chloroethyl)dichloromethylsilane, and (3-chloropropyl)dimethoxymethylsilane were purchased from Petrarch Systems, Levittown, PA, USA and 5-Phenylpentanoic acid was from Aldrich, Milwaukee, WI, USA. Celite No. 535 was a product of Johns-Manville, Denver, CO, USA and 3,5-dinitrophenyl isocyanate was purchased from Sumika Chemical Analysis Service, Osaka, Japan. All other chemicals were also obtained from commercial sources.

### Enzymes

The enzymes used in this study were as follows: Lipase from *Candida antarctica* (Novo Nordisk, Copenhagen, Denmark); lipases from *Candida cylindracea*, A, AK, AY, CE, and PS (Amano Seiyaku, Nagoya, Japan); lipase LKIP-001 (Kurita Kogyo, Tokyo, Japan); lipase OF 360 (Meito Sangyo, Tokyo, Japan); lipase Saiken 100 (Osaka Saikin Kenkyusho, Osaka, Japan); lipase (Steapsin) (Tokyo Kasei, Tokyo, Japan); lipase T-01 (Toyo Jozo, Tokyo, Japan); lipase Type II, Type VII, papain, and subtilisin Carlsberg (Sigma, St. Louis, MO, USA); lipoprotein lipase Type A and cholesterol esterase Type A (Toyobo, Osaka, Japan); thermolysin (Daiwa Kasei, Osaka, Japan). These enzymes were used without further purification. Some of the enzymes were obtained from commercial sources and the others were kindly donated by several companies.

### Preparation of substrates: Ethylmethylphenylsilylmethanol (I)

To a solution of phenylmagnesium bromide in 150 ml dry tetrahydrofuran (THF) prepared from magnesium (2.4 g, 100 mmol) and phenyl bromide (17.3 g, 110 mmol) under an N<sub>2</sub> atmosphere, (chloromethyl)diethoxymethylsilane (15.5 g, 85 mmol) was added dropwise at 0 °C and stirred for 6 h. 10 % NH<sub>4</sub>Cl (100 ml) was added slowly and the mixture was warmed to room temperature. The organic layer was separated and the aqueous layer was extracted with ethyl ether (50 ml x 3). The



combined organic layer was washed with saturated NaCl solution, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated in vacuo. The residue was distilled under reduced pressure (3 mmHg) followed by the fractionation at bp 78-80 °C to give crude (chloromethyl)ethoxymethylphenylsilane as colorless oil (15.5 g, 84 %):  $^1\text{H-NMR}$  (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.53 (s, 3H,  $\text{SiCH}_3$ ), 1.23 (t, 3H,  $J=8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 3.02 (s, 2H,  $\text{SiCH}_2\text{Cl}$ ), 3.82 (q, 2H,  $J=8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 7.3-7.7 (m, 5H,  $\text{SiC}_6\text{H}_5$ ).

(Chloromethyl)ethoxymethylphenylsilane was added dropwise to a solution of ethylmagnesium bromide in 150 ml dry THF prepared from magnesium (3.8 g, 160 mmol) and ethyl bromide (18.5 g, 170 mmol) under an  $\text{N}_2$  atmosphere, and the mixture was refluxed for 8 h. After cooling to room temperature, the mixture was worked up in the same procedure described above and distilled. The fractionation at bp 78-81 °C (3 mmHg) gave crude (chloromethyl)ethylmethylphenylsilane as colorless oil (13.0 g, 90 %):  $^1\text{H-NMR}$  (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.43 (s, 3H,  $\text{SiCH}_3$ ), 0.98 (m, 5H,  $\text{SiC}_2\text{H}_5$ ), 2.98 (s, 2H,  $\text{SiCH}_2\text{Cl}$ ), 7.2-7.6 (m, 5H,  $\text{SiC}_6\text{H}_5$ ); IR (neat) 2950, 1425, 1258, 1115, 1005, 797, 695  $\text{cm}^{-1}$ .

To a stirred mixture of magnesium (1.94 g, 80 mmol) and 100 ml dry THF, (chloromethyl)ethylmethylphenylsilane was added dropwise under an  $\text{N}_2$  atmosphere. Ethyl bromide (0.22 g, 2 mmol) was added as an initiator, and the mixture was refluxed for 2 h and cooled to 0 °C. Dry oxygen gas was slowly introduced into the mixture<sup>16)</sup> followed by addition of 10 %

$\text{NH}_4\text{Cl}$  (80 ml). After stirring for 1 h at room temperature, the organic layer was separated and the aqueous layer was extracted with ethyl ether (50 ml x 3). The combined organic layer was washed with saturated NaCl solution, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was chromatographed on silica gel 60 (No.7734, Merck, Darmstadt, FRG) (mobile phase; *n*-hexane/ethyl ether, 4:1 v/v) and distilled under reduced pressure to give **1** (bp 87-88 °C, 4 mmHg) as colorless oil (8.1 g, 69 %):  $^1\text{H-NMR}$  (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.32 (s, 3H,  $\text{SiCH}_3$ ), 0.8-1.0 (m, 5H,  $\text{SiC}_2\text{H}_5$ ), 1.29 (s, 1H, OH), 3.53 (s, 2H,  $\text{SiCH}_2\text{OH}$ ), 7.1-7.5 (m, 5H,  $\text{SiC}_6\text{H}_5$ ); IR (neat) 3350, 2950, 2870, 1425, 1250, 1110, 1000, 815, 730, 695  $\text{cm}^{-1}$ ; MS,  $m/z$  180 ( $\text{M}^+$ ), 149, 121, 89; Anal. Calcd for  $\text{C}_{10}\text{H}_{16}\text{OSi}$ : C, 66.61; H, 8.94. Found: C, 66.63; H, 8.79.

The other substrates shown in Fig.1 were prepared by the same procedure. **1** and **4-9** were synthesized from chloromethyldiethoxymethylsilane, **2** from (2-chloroethyl)dimethoxymethylsilane, and **3** from (3-chloropropyl)dimethoxymethylsilane. (2-Chloroethyl)dimethoxymethylsilane was prepared from (2-chloroethyl)dichloromethylsilane as described by Leasure and Speier.<sup>17)</sup> Spectral data are given below.

#### **2-(Ethylmethylphenylsilyl)ethanol (2)**

Bp 108-110 °C (3 mmHg);  $^1\text{H-NMR}$  (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.28 (s, 3H,  $\text{SiCH}_3$ ), 0.8-1.0 (m, 5H,  $\text{SiC}_2\text{H}_5$ ), 1.15 (t, 2H,  $J=8$  Hz,  $\text{SiCH}_2\text{CH}_2\text{OH}$ ), 1.61



(s, 1H, OH), 3.66 (t, 2H,  $J=8$  Hz,  $\text{SiCH}_2\text{CH}_2\text{OH}$ ), 7.1-7.5 (m, 5H,  $\text{SiC}_6\text{H}_5$ ); IR (neat) 3320, 2950, 2860, 1425, 1250, 1110, 1035, 790, 730, 695  $\text{cm}^{-1}$ ; MS,  $m/z$  176 ( $\text{M}^+-\text{H}_2\text{O}$ ), 137, 121; Anal. Calcd for  $\text{C}_{11}\text{H}_{18}\text{OSi}$ : C, 67.98; H, 9.34. Found: C, 67.99; H, 9.14.

**3-(Ethylmethylphenylsilyl)propanol (3)**

Bp 125-126 °C (3 mmHg);  $^1\text{H}$ -NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.26 (s, 3H,  $\text{SiCH}_3$ ), 0.6-1.2 (m, 7H,  $\text{SiC}_2\text{H}_5$  and  $\text{SiCH}_2\text{CH}_2\text{CH}_2\text{OH}$ ), 1.3-1.7 (m, 2H,  $\text{SiCH}_2\text{CH}_2\text{CH}_2\text{OH}$ ), 1.90 (s, 1H, OH), 3.46 (t, 2H,  $J=6$  Hz,  $\text{SiCH}_2\text{CH}_2\text{CH}_2\text{OH}$ ), 7.0-7.4 (m, 5H,  $\text{SiC}_6\text{H}_5$ ); IR (neat) 3340, 2950, 2930, 2870, 1425, 1255, 1115, 1055, 1010, 785, 735, 700  $\text{cm}^{-1}$ ; MS,  $m/z$  208 ( $\text{M}^+$ ), 179, 137, 121; Anal. Calcd for  $\text{C}_{12}\text{H}_{20}\text{OSi}$ : C, 69.17; H, 9.67. Found: C, 69.11; H, 9.51.

**Methylphenyl-n-propylsilylmethanol (4)**

Bp 96-98 °C (10 mmHg);  $^1\text{H}$ -NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.33 (s, 3H,  $\text{SiCH}_3$ ), 0.9-1.7 (m, 8H,  $\text{SiC}_3\text{H}_7$  and OH), 3.53 (s, 2H,  $\text{SiCH}_2\text{OH}$ ), 7.1-7.6 (m, 5H,  $\text{SiC}_6\text{H}_5$ ); IR (neat) 3350, 2950, 2860, 1425, 1248, 1110, 1062, 995, 820, 732, 695  $\text{cm}^{-1}$ ; MS,  $m/z$  198 ( $\text{M}^+$ ), 167, 139; Anal. Calcd for  $\text{C}_{11}\text{H}_{18}\text{OSi}$ : C, 67.98; H, 9.34. Found: C, 67.90; H, 9.62.

**n-Hexylmethylphenylsilylmethanol (5)**

Bp 132-133 °C (6 mmHg);  $^1\text{H}$ -NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.31 (s, 3H,

$\text{SiCH}_3$ ), 0.6-1.6 (m, 14H,  $\text{SiC}_6\text{H}_{13}$  and OH), 3.52 (s, 2H,  $\text{SiCH}_2\text{OH}$ ), 7.1-7.6 (m, 5H,  $\text{SiC}_6\text{H}_5$ ); IR (neat) 3335, 2950, 2918, 2850, 1428, 1250, 1113, 1000, 800, 732 698  $\text{cm}^{-1}$ ; MS,  $m/z$  194 ( $\text{M}^+$ ), 163, 135, 89; Anal. Calcd for  $\text{C}_{14}\text{H}_{24}\text{OSi}$ : C, 71.12; H, 10.23. Found: C, 71.19; H, 10.32.

**Ethylmethyl(p-methylphenyl)silylmethanol (6)**

Bp 97 °C (3 mmHg);  $^1\text{H}$ -NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.30 (s, 3H,  $\text{SiCH}_3$ ), 0.7-1.0 (m, 5H,  $\text{SiC}_2\text{H}_5$ ), 1.08 (s, 1H, OH), 2.32 (s, 3H,  $\text{CH}_3$ ), 3.55 (s, 2H,  $\text{SiCH}_2\text{OH}$ ), 7.0-7.5 (m, 4H,  $\text{SiC}_6\text{H}_4$ ); IR (neat) 3350, 2945, 2865, 1600, 1455, 1388, 1245, 1102, 1003, 790  $\text{cm}^{-1}$ ; MS,  $m/z$  194 ( $\text{M}^+$ ), 163, 121; Anal. Calcd for  $\text{C}_{11}\text{H}_{18}\text{OSi}$ : C, 67.98; H, 9.34. Found: C, 67.96; H, 9.55.

**Ethyl(p-fluorophenyl)methylsilylmethanol (7)**

Bp 102-103 °C (3 mmHg);  $^1\text{H}$ -NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  0.32 (s, 3H,  $\text{SiCH}_3$ ), 0.8-1.1 (m, 5H,  $\text{SiC}_2\text{H}_5$ ), 1.23 (s, 1H, OH), 3.56 (s, 2H,  $\text{SiCH}_2\text{OH}$ ), 6.8-7.7 (m, 4H,  $\text{SiC}_6\text{H}_4$ ); IR (neat) 3350, 2948, 2865, 1582, 1495, 1228, 1158, 1100, 1003, 820  $\text{cm}^{-1}$ ; MS,  $m/z$  236 ( $\text{M}^+$ ), 205, 151, 121; Anal. Calcd for  $\text{C}_{10}\text{H}_{15}\text{OFSi}$ : C, 60.57; H, 7.62; F, 9.58. Found: C, 60.47; H, 7.54; F, 9.64.



#### ***Ethyl-n-hexylmethylsilylmethanol (8)***

Bp 94-95 °C (7 mmHg); <sup>1</sup>H-NMR (60 MHz, CDCl<sub>3</sub>) δ 0.02 (s, 3H, SiCH<sub>3</sub>), 0.4-1.5 (m, 19H, SiC<sub>2</sub>H<sub>5</sub>, SiC<sub>6</sub>H<sub>13</sub>, and OH), 3.36 (s, 2H, SiCH<sub>2</sub>OH); IR(neat) 3330, 2950, 2915, 2850, 1427, 1250, 1110, 998, 800, 725, 695 cm<sup>-1</sup>; MS, m/z 188 (M<sup>+</sup>), 129, 73; Anal. Calcd for C<sub>10</sub>H<sub>24</sub>OSi: C, 63.76; H, 12.84. Found: C, 63.65; H, 12.56.

#### ***Ethylmethyl-n-propylsilylmethanol (9)***

Bp 154 °C; <sup>1</sup>H-NMR (60 MHz, CDCl<sub>3</sub>) δ 0.03 (s, 3H, SiCH<sub>3</sub>), 0.5-1.5 (m, 12H, SiC<sub>2</sub>H<sub>5</sub> and SiC<sub>3</sub>H<sub>7</sub>), 1.77 (s, 1H, OH), 3.38 (s, 2H, SiCH<sub>2</sub>OH); IR (neat) 3330, 2948, 2860, 1455, 1248, 1060, 992, 818 cm<sup>-1</sup>; MS, m/z 128 (M<sup>+</sup>-H<sub>2</sub>O), 115, 73; Anal. Calcd for C<sub>7</sub>H<sub>18</sub>OSi: C, 57.47; H, 12.40. Found: C, 57.21; H, 12.39.

#### ***Adsorption of enzyme on Celite***

Enzyme preparation (100 mg) suspended in 100 µl deionized water was mixed thoroughly with 250 mg Celite No. 535.

#### ***Screening of hydrolase***

The reaction mixture for esterification was composed of Celite-adsorbed hydrolase (corresponding to 20 mg enzyme) and 2 ml water-saturated 2,2,4-trimethylpentane containing 100 mM (±)-**1** and 100 mM 5-phenylpentanoic

acid. The reaction was carried out in 15 ml test tube at 30 °C with shaking (120 strokes·min<sup>-1</sup>). The ester was quantitatively determined by GLC using a glass column (diameter 3.0 mm x 1.0 m) packed with silicon SE-30 supported on Chromosorb W AW-DMCS (Nishio Kogyo, Tokyo, Japan) (carrier gas, N<sub>2</sub>; flow rate, 60 ml·min<sup>-1</sup>). *n*-Icosane was used as the internal standard. The optical purity of the remaining alcohols after the reaction was determined with HPLC using two columns of Sumichiral OA-4600 (diameter 4.0 mm x 250 mm, Sumika Chemical Analysis Service) in series after derivatization with 3,5-dinitrophenyl isocyanate.<sup>18)</sup> The mobile phase was *n*-hexane/2-propanol, 98:2 v/v and the flow rate was 1.0 ml·min<sup>-1</sup>. The eluent was monitored at 254 nm. The enantiomeric excess (%ee) was calculated from the peak areas of the both enantiomers.

The reaction mixture for transesterification was composed of 20 mg hydrolase (without adsorption on Celite) and 2 ml water-saturated 2,2,4-trimethylpentane containing 100 mM (±)-**1** and 250 mM vinyl acetate. The ester formed and the optical purity of the remaining alcohols were determined by the same procedures described above.

#### ***Enantioselective esterification catalyzed by crude papain***

Celite-adsorbed crude papain (corresponding to 100 mg enzyme) and 10 ml water-saturated 2,2,4-trimethylpentane containing 100 mM racemic alcohol shown in Fig. 1 and 100 mM 5-phenylpentanoic acid were mixed in



100 ml flask and shaken (120 strokes  $\text{min}^{-1}$ ) at 30 °C. The ester was determined by GLC as described above. The mixture was filtered off to stop the reaction at more than 50 % conversion, and the filtrate was concentrated. The ester and alcohol were isolated by column chromatography on silica gel 60 (mobile phase; *n*-hexane/ethyl ether, 10:1 v/v for the ester and 4:1 v/v for the alcohol). Each enantiomers of **1**, **4**, **5**, **6**, and **7** were separated by HPLC as described above and the optical purity of these alcohols and esters was determined (the esters isolated were reduced to the corresponding alcohols by  $\text{LiAlH}_4$  in dry ethyl ether prior to analysis. No racemization occurred through this treatment). In the cases of **2** and **9**, the optical purity was determined with  $^{19}\text{F}$ -NMR after derivatization with (*S*)-cyanofluorophenyl-acetic acid (CFPA) as reported by Takeuchi *et al.*<sup>19-20</sup> Specific rotation of the alcohols and esters isolated was measured in  $\text{CHCl}_3$  at 20 °C.

## RESULTS AND DISCUSSION

### Screening of hydrolase

Twenty kinds of commercially available hydrolases containing lipases, a lipoprotein lipase, a cholesterol esterase, and proteases from various sources were examined to screen highly active and enantioselective enzymes towards **1** on esterification with 5-phenylpentanoic acid in water-saturated 2,2,4-trimethylpentane (Table 1). Many of the hydrolases exhibited the esterification

activity except for lipases from bacteria and proteases from *Bacillus* sp., but the optical purity of remaining **1**, determined by HPLC, was not so high as the whole. While there has been a much of successful reports for kinetic resolution of racemic secondary alcohols with hydrolases,<sup>21-22</sup> the chiral recognition of primary alcohols was generally difficult for both enzymatic and chemical methods due to the smaller bulkiness around the hydroxy group compared to secondary alcohols. In fact, many hydrolases showed only a low enantioselectivity toward **1** despite of their esterification activity. Only one enzyme, a commercial crude papain preparation, was found to exhibit a high enantioselectivity and moderate activity toward **1**, resulting in highly optically active (+)-**1** (92 %ee). Although there were several hydrolases which could preferentially esterify the (+)-enantiomer (lipases from animal, a lipoprotein lipase, and a cholesterol esterase), the high opposite enantioselectivity was never observed under the conditions employed.

Enantioselective transesterification of **1** with vinyl acetate in water-saturated 2,2,4-trimethylpentane was also tried (Table 2). Vinyl acetate and related enol esters are very useful acyl donors because the enols produced after transesterification rapidly tautomerize to the corresponding aldehydes or ketones, thus preventing the back reactions.<sup>23</sup> Fifteen kinds of hydrolases were tested and thirteen hydrolases, containing lipases from bacteria, exhibited the transesterification activity toward **1**. However, high optical purity of remaining **1** was not obtained; 49 %ee showed by crude papain was the



Table 1. Screening of hydrolase for enantioselective esterification of ethylmethylphenylsilylmethanol (1) with 5-phenylpentanoic acid in organic solvent

Hydrolase	Source	Time (h)	Conv. <sup>a</sup> (%)	%ee <sup>b</sup>	Optical <sup>c</sup> activity
Lipase	<i>Candida antarctica</i>	32	12	1	
Lipase	<i>Candida cylindracea</i>	11	45	10	(+)
Lipase AY	<i>Candida rugosa</i>	45	44	0	
Lipase OF 360	<i>Candida cylindracea</i>	12	56	14	(+)
Lipase Type VII	<i>Candida cylindracea</i>	25	61	3	
Lipase A	<i>Aspergillus niger</i>	143	34	2	
Lipase CE	<i>Humicola langinosa</i>	94	60	22	(+)
Lipase Saiken 100	<i>Rhizopus japonicus</i>	25	50	47	(+)
Lipase AK	<i>Pseudomonas</i> sp.	94	2	-	
Lipase LKIP-001	<i>Pseudomonas</i> sp.	24	0	-	
Lipase PS	<i>Pseudomonas</i> sp.	94	2	-	
Lipase T-01	<i>Chromobacterium viscosum</i>	24	0	-	
Lipase(Steapsin)	Hog pancreas	15	51	23	(-)
Lipase Type II	Hog pancreas	94	46	10	(-)
Lipase Type II	Porcine pancreas	11	43	14	(-)

Table 1. (Continued)

Lipoprotein lipase	<i>Pseudomonas</i> sp.	4	48	25	(-)
Type A					
Cholesterol esterase	<i>Pseudomonas</i> sp.	16	54	26	(-)
Type A					
Papain	<i>Papaya latex</i>	36	60	92	(+)
Subtilisin Carlsberg	<i>Bacillus licheniformis</i>	72	2	-	
Thermolysin	<i>Bacillus thermoproteolyticus</i>	72	2	-	

The reaction was carried out with 5-phenylpentanoic acid and 20 mg hydrolase adsorbed on Celite in water-saturated 2,2,4-trimethylpentane.

<sup>a</sup> Conversion ratio determined by GLC.

<sup>b</sup> %ee of the remaining alcohols determined by HPLC.

<sup>c</sup> Optical activity of the remaining alcohols

Table 2. Screening of hydrolase for enantioselective transesterification of **1** with vinyl acetate in organic solvent

Hydrolase	Time (h)	Conv. <sup>a</sup> (%)	%ee <sup>b</sup>	Optical <sup>c</sup> activity
Lipase AY	1	67	10	(-)
Lipase OF 360	2	56	4	
Lipase Type VII	2	52	0	
Lipase A	147	50	5	(+)
Lipase CE	147	44	1	
Lipase Saiken 100	168	13	1	
Lipase AK	120	50	4	(-)
Lipase PS	48	64	6	
Lipase (Steapsin)	168	28	2	
Lipase Type II	144	16	0	
Lipoprotein lipase	71	46	7	(-)
Cholesterol esterase	1	76	23	
Papain	144	54	49	(+)
Subtilisin Carlsberg	96	3	-	
Thermolysin	96	0	-	

The reaction was carried out with vinyl acetate and 20 mg hydrolase in water-saturated 2,2,4-trimethylpentane.

<sup>a</sup> Conversion ratio determined by GLC.

<sup>b</sup> %ee of the remaining alcohols determined by HPLC.

<sup>c</sup> Optical activity of the remaining alcohols.

highest value in the series of this experiment.

Thus, the enantioselective esterification with 5-phenylpentanoic acid catalyzed by crude papain was selected for further study because of the high enantioselectivity toward **1**.

#### *Effect of chain length between the hydroxyl group and the silicon atom*

The chain length between the hydroxyl group and the silicon atom was changed from 1 to 3 (**1-3**), and its effect on the activity and enantioselectivity of crude papain was investigated. It is clearly shown in Table 3 that **1** was the most reactive among the three substrates examined and that the reaction rate became lower with increasing the chain length. A similar phenomenon was also observed in the case of Me<sub>3</sub>Si(CH<sub>2</sub>)<sub>n</sub>OH (n=1-3) which were used as acyl acceptors on lipase-catalyzed esterification of 2-(4-chlorophenoxy)propanoic acid.<sup>3</sup> One of the factor that caused these phenomena supposed to be the specific character of the silicon atom, that is, the silicon atom in these alcohols increases the nucleophilicity of the hydroxyl oxygen atom owing to its low electronegativity, but such activation becomes weaker with the increase in distance of the hydroxyl group from the silicon atom.

The optical purity of the alcohols and esters is also shown in Table 3. Compound **1** was recovered with high optical purity (92 %ee) at 58 % conversion, while the optical purity of the ester produced was 67 %ee,



Table 3. Effect of chain length between the hydroxyl group and the silicon atom on enantioselective esterification catalyzed by crude papain preparation with 5-phenylpentanoic acid

Alcohol	PhMeEtSi(CH <sub>2</sub> ) <sub>n</sub> OH		Time (h)	Conv. (%)	Alcohol		Ester	
	n				%ee	[α] <sub>D</sub> <sup>a</sup>	%ee	[α] <sub>D</sub> <sup>a</sup>
<b>1</b>	1		38	58 <sup>b</sup>	92 <sup>c</sup>	+5.7	67 <sup>e</sup>	-2.2
<b>2</b>	2		62	59 <sup>b</sup>	62 <sup>d</sup>	+1.2	43 <sup>d</sup>	-1.1
<b>3</b>	3		186	53 <sup>e</sup>	0 <sup>f</sup>	0.0	0 <sup>f</sup>	0.0

The reaction was carried out with 5-phenylpentanoic acid and 100 mg crude papain adsorbed on Celite in water-saturated 2,2,4-trimethylpentane.

<sup>a</sup> Specific rotation measured at 20 °C (w/v) in CHCl<sub>3</sub>.

<sup>b</sup> Conversion ratio determined from %ee of the alcohols and esters.

<sup>c</sup> %ee determined by HPLC.

<sup>d</sup> %ee determined by <sup>19</sup>F-NMR after derivatization with (S)-CFPA.<sup>19-20)</sup>

<sup>e</sup> Conversion ratio determined by GLC.

<sup>f</sup> %ee estimated from the specific rotation.

indicating the incomplete enantioselectivity of crude papain toward this alcohol. In the case of **2**, the optical purity determined by <sup>19</sup>F-NMR after derivatization with (S)-CFPA<sup>19-20)</sup> was 62 %ee for the alcohol and 43 %ee for the ester. The enantioselectivity of the enzyme toward **3** was, furthermore, negligible or very low based on the specific rotation of the alcohol and ester, both being zero. Clearly, the enantioselectivity of crude papain toward **1-3** decreased upon increasing the chain length between the hydroxyl group and the silicon atom. The enantiomeric recognition became more difficult for the enzyme with increasing the distance of the functional group from the chiral center.

The high reaction rate was inconsistent with the high enantioselectivity as observed with lipase-catalyzed esterification using conventional compounds as substrates,<sup>21, 24)</sup> but this experience was not correct in the case of unconventional substrates, organosilicon compounds. The more reactive hydroxysilanes having a shorter methylene chain could be resolved with higher enantioselectivity by crude papain.

#### Effect of the substituent groups attached to the silicon atom

Various silylmethanol derivatives, that is, analogues of **1** having different substituent groups instead of the phenyl or ethyl group on the silicon atom, R<sup>1</sup>R<sup>2</sup>MeSiCH<sub>2</sub>OH (**4-9**), were synthesized as racemic compounds and examined for the enantioselective esterification catalyzed

by crude papain (Table 4). When R<sup>1</sup> was the phenyl group and R<sup>2</sup> was an *n*-alkyl group (**1**, **4**, and **5**), the reaction rate of the esterification decreased with the increase in chain length of R<sup>2</sup> (38 h for **1**, 137h for **4**, and 456 h for **5** until 54-58 % conversion), probably due to the increased steric hindrance at R<sup>2</sup> position. Although the enantioselectivity of the enzyme toward **4** was still as high as that toward **1**, the optical purity of the alcohol and the ester was very low in the case of **5** (34 and 30 %ee, respectively). Compound **6**, which had a *p*-methylphenyl group instead of the phenyl group, was esterified slower, but the optical purity of remaining **6** (96 %ee) was higher than that of **1**. These results suggested that steric hindrance around the silicon atom decreased the reaction rate, but that difference of the bulkiness between R<sup>1</sup> and R<sup>2</sup> would be necessary for crude papain to recognize the chirality of the silyl-methanol derivatives.

Introduction of a fluorine atom at *p*-position of the benzene ring (**7**) enhanced the reactivity. Due to the electron-attracting fluorine atom, the molecule would be more strongly polarized and the nucleophilicity of the oxygen atom would be increased in this case, resulting in the higher reactivity of **7** than that of **1**. This substitution increased not only the reactivity but also the enantioselectivity of the enzyme; the optical purity of remaining **7** reached 99 %ee. In this reaction system, **7** was the most efficiently resolved compound among the silicon-containing alcohols used here.

Drastic decrease of the reaction rate was observed when a linear

Table 4. Effect of substitution groups attached to the silicon atom on enantioselective esterification catalyzed by crude papain preparation with 5-phenylpentanoic acid

	R <sup>1</sup> R <sup>2</sup> MeSiCH <sub>2</sub> OH		Time (h)	Conv. <sup>a</sup> (%)	Alcohol		Ester	
	Alcohol	R <sup>1</sup>	R <sup>2</sup>		%ee <sup>b</sup>	[α] <sub>D</sub> <sup>c</sup>	%ee <sup>b</sup>	[α] <sub>D</sub> <sup>c</sup>
<b>1</b>		Ph	Et	38	58	+5.7	67	-2.2
<b>4</b>		Ph	Pr	137	58	+1.6	64	-1.1
<b>5</b>		Ph	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	456	54	+1.4	30	-0.7
<b>6</b>		<i>p</i> -Me-Ph	Et	72	59	+5.4	68	-1.6
<b>7</b>		<i>p</i> -F-Ph	Et	28	59	+5.5	70	-2.2
<b>8</b>		<i>n</i> -C <sub>6</sub> H <sub>13</sub>	Et	384	60	+0.2	27 <sup>d</sup>	-0.1
<b>9</b>		Pr	Et	963	24 <sup>e</sup>	-	-	-

The reaction was carried out with 5-phenylpentanoic acid and 100 mg crude papain adsorbed on Celite in water-saturated 2,2,4-trimethylpentane.

<sup>a</sup> Conversion ratio determined from %ee of the alcohols and esters.

<sup>b</sup> %ee determined by HPLC.

<sup>c</sup> Specific rotation measured at 20 °C (w/v) in CHCl<sub>3</sub>.

<sup>d</sup> %ee determined by <sup>19</sup>F-NMR after derivatization with (S)-CFPA.<sup>19-20</sup>

<sup>e</sup> Conversion ratio determined by GLC.



*n*-alkyl group was substituted for the phenyl group (**8** having *n*-C<sub>6</sub>H<sub>13</sub> and **9** having Pr at R<sup>1</sup> position). The reaction time required to achieve 60 % conversion was 384 h for **8**, that is, 10 times longer compared to **1**. In the case of **9**, the conversion ratio was only 24% after 963 h and never reached 50 % even after prolonged reaction time. The optical purity of remaining **8** and the corresponding ester determined by <sup>19</sup>F-NMR using (*S*)-CFPA was only 40 %ee and 27 %ee at 60 % conversion, respectively. Clearly, the aromatic ring on the silicon atom was essential to express both the high esterification activity and high enantioselectivity for the enzyme. Interaction between the aromatic ring and a binding pocket of the enzyme would play important roles in incorporation of the substrates and recognition of the chirality through the reaction.

As a result, several kinds of highly optically active silylmethanol derivatives having an asymmetric silicon atom (**1**, **4**, **6**, and **7**) were successfully obtained, and other related silanes will be also resolved by using this reaction system. It is generally difficult to prepare such chiral quaternary silanes with high optical purity by chemical methods.<sup>8-9)</sup> Chemical kinetic resolution and asymmetric synthesis require leaving groups attached to the silicon atom, and furthermore, optical purity attained is not high in these cases. Stereospecific substitution of optically active halogenosilanes with carbon nucleophiles is a potent method for preparing the chiral quaternary silanes, but synthesis of the chiral halogenosilanes with desired structure is

very complicated. However, the enzymatic kinetic resolution enables the convenient preparation of such chiral quaternary silanes from the racemic compounds which are easily synthesized by traditional methodology.

Recent research has developed a biologically active quaternary silane ([[(1,2,4-triazol-1-yl)methyl]silane, Flusilazole) as a fungicidal agrochemical.<sup>14,25)</sup> The optically active silylmethanols prepared by the crude papain-catalyzed enantioselective reaction, for example, will be applicable for the synthesis of chiral analogues of such useful quaternary organosilicon compounds.

In conclusion, this study has revealed that enzymes can recognize the chirality not only on the carbon atom but also on the silicon atom, and this fact indicates the usefulness of enzymes for preparing optically active silanes.

## SUMMARY

Kinetic resolution of ethylmethylphenylsilylmethanol, a primary alcohol having an asymmetric silicon atom, was tried by hydrolase-catalyzed enantioselective reactions. Among twenty kinds of hydrolases examined, a commercial crude papain preparation was found to exhibit the highest enantioselectivity with moderate activity toward the silicon-containing alcohol on the esterification with 5-phenylpentanoic acid in an organic solvent system, and 92 %ee of the (+)-enantiomer could be obtained as the remaining



substrate. This enzyme could also resolved several silylmethanol derivatives by the enantioselective esterification, even though it was difficult to synthesize such chiral quaternary silanes with high optical purity by chemical methods due to the absence of leaving groups on the silicon atom. A short methylene chain between the silicon atom and the hydroxyl group, and an aromatic substituent on the silicon atom were essential to achieve the high activity and high enantioselectivity with this system. These results demonstrate that enzymes can recognize the chirality not only on the carbon atom but also on the silicon atom, and indicate the usefulness of biocatalysts for preparing optically active silanes.

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## GENERAL CONCLUSION

The present study deals with the bioconversion of organosilicon compounds by an alcohol dehydrogenase and hydrolases. Because organosilicon compounds show unique chemical and physical properties due to the specific characteristics of the silicon atom, the author selected them as unconventional substrates to investigate the behavior of enzymes toward non-natural compounds.

First, trimethylsilylalkanols ( $\text{Me}_3\text{Si}(\text{CH}_2)_n\text{OH}$ ,  $n=1-3$ ) were examined as substrates for horse liver alcohol dehydrogenases (HLADH), and it was found that the specific properties of the silicon atom greatly affected the dehydrogenation activity of HLADH. On the reaction of 2-trimethylsilyl-ethanol, the  $\beta$ -effect of the silicon atom made remove of the  $\text{H}^-$  anion, the first step of the dehydrogenation, from the  $\beta$ -carbon atom easier in comparison with that of its carbon analogue. This easier remove of the  $\text{H}^-$  anion, that is, the lower activation energy of the reaction resulted in the improved activity of HLADH toward the organosilicon compound. Another factor for the improved activity was the higher affinity of HLADH toward 2-trimethylsilyl-ethanol probably owing to higher hydrophobicity of the trimethylsilyl group, because HLADH was known to have a hydrophobic active center. In contrast, the inactivity of HLADH toward trimethylsilylmethanol was explained by the assumption that the electric effect of the silicon atom, which can stabilize



the negative charge on the  $\alpha$ -carbon atom, made it difficult for the  $H^-$  anion to be removed from the  $\alpha$ -carbon atom.

The silicon atom in substrates was found to affect not only the activity but also the enantioselectivity of HLADH. This enzyme could enantioselectively dehydrogenate racemic 1-trimethylsilyl-2-propanol and 2-trimethylsilyl-1-propanol in a water-*n*-hexane two-layer system with coenzyme regeneration using L-glutamate dehydrogenase and 2-oxoglutarate. The silicon substitution for the carbon atom enhanced the enantioselectivity of HLADH. The more bulky and/or hydrophobic trimethylsilyl group derived from the bigger atomic radius of the silicon atom than that of the carbon atom supposed to be favorable for the enzyme to recognize the difference of enantiomers. Especially, highly optically pure (*R*)-(-)-1-trimethylsilyl-2-propanol (>99 %ee) could be obtained.

The author successfully established a novel *in situ*  $NAD^+$  regeneration system utilizing a specific property of organosilicon compounds. In enantioselective dehydrogenation of primary  $\beta$ -hydroxysilanes with HLADH,  $NAD^+$  was proved to be regenerated through HLADH-catalyzed reduction of aliphatic aldehydes produced by spontaneous degradation of the dehydrogenated products,  $\beta$ -carbonylsilanes, so the  $\beta$ -hydroxysilanes could be resolved with only HLADH and a catalytic amount of  $NAD^+$ . Advantages of this *in situ* coenzyme regeneration are simplicity of the reaction system without any other enzymes and substrates for the regeneration, and no

product inhibition due to the degradation of the dehydrogenated products. These results indicate that novel bioconversion systems can be constructed by applying the specific characters of organosilicons. Furthermore, it was found that enough difference of bulkiness and/or hydrophobicity among the substituents around the chiral center was important for HLADH to express the high enantioselectivity toward primary alcohols.

Second, hydrolases were used as catalysts for enantioselective conversion of organosilicon compounds. Because of their hydrophobic character, the condensation activity of hydrolases in organic solvents was utilized for the efficient conversion. Enantioselective esterification of three isomers of racemic trimethylsilylpropanols were examined with five kinds of hydrolases, and the enzymes could esterify them with 5-phenylpentanoic acid, even  $\beta$ -hydroxysilanes which are unstable under the conditions of acid-catalyzed esterification. The highly optically pure silicon-containing alcohols, including a primary alcohol of which conversion with high stereoselectivity was generally difficult, were successfully prepared by using selected hydrolases. Comparative studies revealed that the hydrolases often showed higher enantioselectivity toward the silicon compounds than toward their corresponding carbon compounds, probably due to the greater bulkiness of the trimethylsilyl group, being similar to the case of HLADH. The specific properties of the silicon atom also affected the reactivity of the substrates, but the effect was dependent on their structure.



Chemoenzymatic preparation of optically active silylmethanol derivatives having an asymmetric silicon atom was carried out by using hydrolase-catalyzed enantioselective esterification as a key reaction. A commercial crude papain preparation was screened among twenty kinds of hydrolases because it exhibited the highest enantioselectivity with moderate activity toward chemically synthesized racemic ethylmethylphenylsilylmethanol on esterification with 5-phenylpentanoic acid, giving 92 %ee of the (+)-enantiomer. A short methylene chain between the silicon atom and the hydroxyl group, and an aromatic ring and two different small alkyl groups attached to the silicon atom were essential for the efficient kinetic resolution, and several silylmethanol derivatives could be resolved with high optical purity by this reaction system in spite of the difficulty in chemically preparing such chiral quaternary silanes. Enzymes were revealed to be able to recognize the chirality not only on the carbon atom but also on the silicon atom.

The study summarized here not only gives a clue to further research on the recognition and reaction of enzymes, but also provides applicable procedures for preparation of various optically active organosilicon compounds with enzymes. The author believes that this study will increase the potential of and expand the application of enzymes.

## PUBLICATION LIST

### PART I

#### Chapter 1.

- 1) Bioconversion of organosilicon compounds by horse liver alcohol dehydrogenase: The role of the silicon atom in enzymatic reactions.  
*Appl. Microbiol. Biotechnol.*, **36**, 40-43 (1991)

#### Chapter 2.

- 2) Kinetic resolution of organosilicon compounds by stereoselective dehydrogenation with horse liver alcohol dehydrogenase.  
*Appl. Microbiol. Biotechnol.*, **38**, 209-213 (1992)

#### Chapter 3.

- 3) Enantioselective dehydrogenation of  $\beta$ -hydroxysilanes catalyzed by horse liver alcohol dehydrogenase with a novel *in situ* NAD<sup>+</sup> regeneration system.  
*Appl. Microbiol. Biotechnol.*, in press

## PART II

### Chapter 1.

- 4) Efficient kinetic resolution of organosilicon compounds by stereoselective esterification with hydrolases in organic solvent.

*Appl. Microbiol. Biotechnol.*, **38**, 482-486 (1993)

- 5) Bioconversion of nonnatural organic compounds: Esterification and dehydrogenation of organosilicon compounds.

*Ann. N. Y. Acad. Sci.*, **672**, 431-435 (1992)

### Chapter 2.

- 6) Chemoenzymatic preparation of optically active silyl-methanol derivatives having an asymmetric silicon atom by hydrolase-catalyzed enantioselective esterification.

in contribution

- 7) Enantioselective bioconversion of non-natural compounds.

in contribution

## Other Publications

- 1) Stereoselective esterification of halogen-containing carboxylic acids by lipase in organic solvent: Effects of alcohol chain length.

*Appl. Microbiol. Biotechnol.*, **34**, 47-51 (1990)

- 2) Long-term continuous production of optically active 2-(4-chlorophenoxy)propanoic acid by yeast lipase in an organic solvent system.

*Appl. Microbiol. Biotechnol.*, **34**, 330-334 (1990)

- 3) Construction of non-support bioreactor: Optical resolution of 2-(4-chlorophenoxy)propanoic acid in an organic solvent system.

*Appl. Microbiol. Biotechnol.*, **35**, 563-567 (1991)